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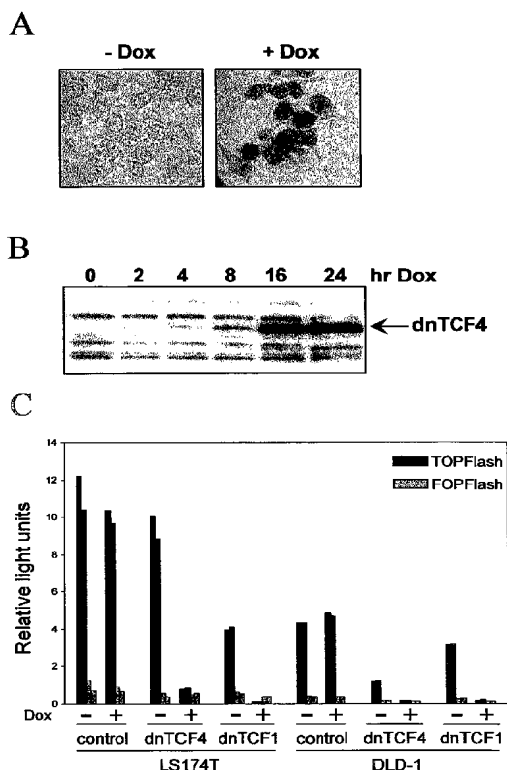
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(54) Title: THE USE OF SPECIFIED TCF TARGET GENES TO IDENTIFY DRUGS FOR THE TREATMENT OF CANCER, IN PARTICULAR COLORECTAL CANCER, IN WHICH TCF/ β -CATENIN/WNT SIGNALLING PLAYS A CENTRAL ROLE



(57) Abstract: The present invention relates to the use of inhibitors of the expressed proteins of TCF target genes whose expression is regulated by TCF/ β -catenin complexes for the preparation of a therapeutic composition for the treatment of cancers in which TCF/ β -catenin signalling is deregulated. Such inhibitors can be antibodies, small molecules, antisense RNA and dsRNA for use in RNAi. The invention also relates to these inhibitors per se.



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THE USE OF SPECIFIED TCF TARGET GENES TO IDENTIFY DRUGS FOR
THE TREATMENT OF CANCER, IN PARTICULAR COLORECTAL CANCER,
IN WHICH TCF/ β -CATENIN/WNT SIGNALLING PLAYS A CENTRAL ROLE

5 The present invention relates to the use of genes
whose expression is regulated by TCF/ β -catenin complexes in
colon carcinoma cells, for the identification and
development of small molecule inhibitors, antibodies,
antisense molecules, RNA interference (RNAi) molecules and
10 gene therapies against these target genes and/or their
expression product, for the treatment of cancer in which
deregulated TCF/ β -catenin signalling occurs, in particular
colorectal cancer and melanomas. In addition the invention
relates to a method for the development of the small
15 molecule inhibitors and antibodies. The invention also
relates to the small molecule inhibitors, antibodies,
antisense molecules, RNAi molecules and therapeutic genes
per se and to their use in the treatment and diagnosis of
cancer in which deregulated TCF/ β -catenin/WNT signalling
20 occurs and to pharmaceutical compositions comprising them.

 The colorectal mucosa contains large numbers of
invaginations known as the crypts of Lieberkühn. Epithelial
cells in these structures are constantly renewed in a
coordinated series of events comprising proliferation, cell
25 migration and differentiation along the crypt axis towards
the intestinal lumen. Pluripotent stem cells are believed
to reside at the bottom positions of the crypt. From these
stem cells, progenitors are generated that occupy the lower
third of the crypt, the amplification compartment. Cells in
30 this compartment divide approximately every 12 hours until
their migration brings them to the mid-crypt region. Here,
they cease proliferating and differentiate into one of the
functional cell types of the colon. At the surface

epithelium, cells undergo apoptosis and/or extrusion into the lumen. The complete process takes approximately 3-5 days.

Colorectal cancer (CRC) is one of the most common malignancies in the western world. The transition of an intestinal epithelial cell into a fully transformed, metastatic cancer cell is a slow process, requiring the accumulation of mutations in multiple proto-oncogenes and tumour suppressor genes. The APC gene, originally cloned from patients with the rare genetic disorder Familial Adenomatous Polyposis, is mutated in the vast majority of sporadic CRCs.

The APC protein resides in the so-called destruction complex, together with GSK3 β , axin/conductin and β -catenin. In this complex, phosphorylation by GSK3 β targets β -catenin for ubiquitination and destruction by the proteasome. Signalling by the extracellular factor WNT inhibits GSK3 β activity. As a result, β -catenin accumulates in the nucleus where it binds members of the TCF family and converts these WNT effectors (from transcriptional repressors into transcriptional activators. The terms "TCF/ β -catenin signalling" and "WNT-signalling" are commonly used to describe the same signalling pathway.

In cancer, truncating mutations in APC and axin/conductin, as well as mutations in the GSK3 β -target residues in β -catenin all lead to the formation of constitutive nuclear β -catenin/TCF complexes. Activating mutations of the WNT pathway are the only known genetic alterations present in early premalignant lesions in the intestine, such as aberrant crypt foci and small polyps. Thus, these mutations appear to initiate the transformation of colorectal epithelial cells.

In the intestinal epithelium, TCF4 is the most prominently expressed TCF family member. Gene disruption in the murine germ line has revealed that during embryonic development TCF4 is required to establish the proliferative progenitors of the prospective crypts in the small intestine.

To better understand the contribution of constitutive β -catenin/TCF activity to the colorectal transformation process, the present inventors have undertaken a large-scale analysis of the downstream genetic program activated by β -catenin/TCF in CRC cells.

During this research it was found that inhibition of β -catenin/TCF activity in fully malignant colorectal cancer cells causes these cells to arrest in G1. DNA array analysis revealed the down-regulation of a small set of transcripts. These genes were expressed in polyps, but also in the normal proliferative compartment of colon crypts. The presence of nuclear β -catenin in this compartment was demonstrated, suggesting that WNT signaling is controlling the self-renewing amplification compartment in the adult intestine. In addition, the induction of multiple marker genes of intestinal differentiation upon inhibition of β -catenin/TCF in CRC cells was observed. It was also found that the cell cycle inhibitor p21^{CIP1/WAF1} is an important mediator of this effect. It was concluded that β -catenin/TCF inhibits differentiation and imposes a crypt progenitor-like phenotype on CRC cells.

Moreover, disruption of β -catenin/TCF-activity in CRC cells was shown to restore the physiological program of epithelial differentiation, despite the presence of multiple other mutations present in these cells.

Thus, a group of target genes was identified whose expression is regulated by TCF/ β -catenin complexes. In colon carcinoma cells, TCF/ β -catenin signalling is deregulated and the resulting inappropriate expression of these target genes is considered to promote carcinogenesis. The transactivation of TCF target genes induced by mutations in APC or β -catenin is believed to represent the primary transforming event in colorectal cancer.

The identification of the target genes of the TCF/ β -catenin signalling pathway provides the opportunity to develop therapeutical compounds or therapies that restore or neutralize the inappropriate expression of these genes when TCF/ β -catenin signalling is deregulated. By normalizing the expression pattern of one or more of the target genes the drugs can halt or reverse the further development of existing cancer cells, such as colon carcinoma cells, for example by the induction of differentiation of the cancer cells, thus restoring the normal cycle of events.

The interference in the inappropriate expression of the target genes can be achieved via the expressed proteins and/or via the transcripts of the genes. These two ways require different active molecules as will be explained herein below.

According to a first aspect, the present invention relates to the use of these target genes and/or their expression products for the development of therapeutical compounds, in particular antibodies, small molecules, antisense molecules and/or RNAi molecules, and gene therapies for treating cancers in which TCF/ β -catenin/WNT signalling is deregulated, in particular colorectal cancer and melanomas.

This is achieved according to a first embodiment of the invention by characterizing the expression product of the target gene and the production of antibodies against the expressed proteins or peptides derived therefrom and/or of small molecules that bind the expressed protein in a way that inhibits or abrogates its biological function.

According to a second embodiment, the target gene sequence information is used to design antisense molecules, RNAi molecules or gene therapies.

10 A further aspect of the present invention relates to the use of the target genes or their expression products for the development of reagents for diagnosis of cancers in which TCF/ β -catenin/WNT signalling is deregulated.

The target genes that were identified according to the invention are the following: CD44, KIT, G protein-coupled receptor 49 (GPR49), Solute Carrier Family 12 member 2 (SLC12A2), Solute Carrier Family 7 member 5, Claudin 1 (CLDN1), SSTK serine threonine kinase, FYN oncogene, EPHB2 receptor tyrosine kinase, EPHB3 receptor tyrosine kinase, EPHB4 receptor tyrosine kinase, ETS2, c-Myc, MYB, ID3, POLE3, Bone Morphogenetic Protein 4 (BMP4), Kit ligand (KITLG), GPX2, GNG2, CDCA7, ENC1, the gene identified with Celera ID hCG40185, the gene identified with Celera ID hCG1645335, the gene represented by IMAGE clone 1871074, the gene identified with Celera ID hCG27486, the gene represented by IMAGE clone 294873, the gene represented by IMAGE clone 940994, the gene identified with Celera ID 39573, the gene represented by IMAGE clone 753028, the gene identified with Celera ID hCG37727, the gene identified with Celera ID hCG40978, and the gene identified with Celera ID hCG1811066. Table 1 gives an overview of these target genes. The sequences of these target genes and their expression products are given in the

figures. According to the present invention, target genes which are preferably used comprise a cDNA sequence as shown in Figures 17-24, preferably a sequence, which is at least 90% homologous to the sequences as shown in the Figures 17-24. In addition, the expressed proteins according to the invention preferably comprise a protein sequence as shown in Figures 17-24, preferably a sequence which is at least 90% homologous to the protein sequences shown in Figures 17-24.

Based on the above TCF/ β -catenin target genes, novel therapeutic compounds and therapies are developed for the treatment of cancer, in particular colorectal cancer and melanomas. Such therapeutic compounds are preferably antibodies, small molecule inhibitors, antisense molecules or RNAi molecules. In addition gene therapies are provided.

Such gene therapies are based on the generation of dominant-negative (dn) forms of the target genes, which inhibit the function of their wild-type counterparts following their directed expression in a cancer cell.

Promoters for use in gene therapy that are specifically activated by TCF/ β -catenin to drive specific expression of dominant-negative or suicide genes in cancer cells with active TCF/ β -catenin signalling are known from e.g. Lipinski et al. (Mol. Ther. 2001 4:365 - High level β -catenin/TCF dependent transgene expression in secondary colorectal cancer tissue), Chen & McCormick (Cancer Res. 2001 61:4445 - Selective targeting to the hyperactive β -catenin/TCF factor pathway in colon cancer cells), and Fuerer & Iggo (Gene Ther. 2002 9:270 - Adenoviruses with TCF-binding sites in multiple early promoters show enhanced selectivity for tumor cells with constitutive activation of the Wnt signalling pathway).

In table 1, genes identified as target of TCF/ β -catenin signalling are summarized. Individual target genes are identified by their official gene symbol and name (unless otherwise stated) as approved by the Human Gene Nomenclature Committee (HGNC), their gene (hCG), Transcript (hCT), Protein (hCP), identification code as referred to in the Celera Discovery System Database (www.celeradiscoverysystem.com) [or their Genbank mRNA and protein ID where stated] and their chromosome localization.

The genes are classified into broad functional groups according to their proposed function. Table 1 also shows the magnitude of down-regulation of expression levels following inhibition of TCF/ β -catenin signalling through expression of dominant-negative TCF-4 (dnTCF4) in LS174T colon carcinoma cells (ND: not determined).

Table 1

Human Gene Symbol/ Clone ID	Human Gene Name	Celera Gene ID	Celera Transcript ID	Celera Protein ID	Chromo-Some	Group	LS174T dnTGF4 fold down-regulation		confirmed by Northern
							11 hr	23 hr	
CD44	CD44 antigen	hCG1811182	hCT1951772	hCP1753227	11	Membrane (Receptor)	1.8	3.2	Yes
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue	hCG22160	hCT13253	hCP39638	4	Membrane (Receptor)	ND	ND	Yes
GPR49	G protein-coupled receptor 49	hCG23766	hCT14878	hCP42243	12	Membrane (Receptor)	2.3	3.6	Yes
SLC12A2	Solute carrier family 12, member 2	hCG27034	hCT18167	hCP44322	5	Membrane	1.8	4.2	Yes
SLC7A5	Solute carrier family 7, member 5	hCG1789357	hCT1828603	hCP1742844	16	Membrane	1.5	3.6	Yes
CLDN1	Claudin 1	hCG17574	hCT8625	hCP33694	3	Membrane	1.2	3.0	ND
SSTK	Serine/threo-nine protein kinase	hCG1640387	hCT1640514	hCP1611903	19	Kinase	1.1	5.6	ND
FYN	Fyn oncogene related to SRC, FGR, YES	hCG34806	hCT26018	hCP47031	6	Kinase	1.3	2.7	ND
EPHB2	Eph-related receptor tyrosine kinase B2	hCG1812037	hCT1955735	hCP1765674	1	Kinase	1.8	3.0	Yes
EPHB3	Eph-related receptor tyrosine kinase B3	hCG16039	hCT7881	hCP33573	3	Kinase	2.9	6.6	Yes

EPHB4	Eph-related receptor tyrosine kinase B4	hCG20448	hCT11528	hCP38155	7	Kinase	ND	ND	Yes
ETS2	v-ets erythroblas- tosis virus E26 oncogene homologue 2 (avian)	hCG401219	hCT401223	hCP801143	21	Trans- cription	1.5	3.4	Yes
c-Myc	v-myc myelocytoma- tosis viral oncogene homologue (avian)	hCG15917	hCT6947	hCP33451	8	Trans- cription	1.5	3.3	Yes
MYB	v-myb myeloblastosis viral oncogene homologue (avian)	hCG32380	hCT23568	hCP46310	6	Trans- cription	1.6	3.3	Yes
ID3	Inhibitor of DNA binding 3, dominant negative helix- loop-helix protein	hCG1739237	hCT1955731/hCT 1955730/hCT195- 5731/hCT177731 9	hCP1765651/hC P1765654/hCP1 713380	1	Trans- cription	2.2	2.9	Yes
POLE3	Polymerase (DNA directed), epsilon 3 (p17 subunit)	hCG29189	hCT20349	hCP44379	9	Trans- cription	ND	ND	ND
BMP4	Bone morphogenetic protein 4	hCG20967	hCT1955929/hCT 12051	hCP1765419/hC P40076	14	Secreted Factor	3.0	3.6	Yes
KITLG	KIT Ligand	hCG26603	hCT18442/hCT18 443	hCP42895/hCP4 2896	12	Secreted Factor/ membrane	ND	ND	Yes
GPX2	Glutathione peroxidase 2 (gastro- intestinal)	hCG40439	hCT31698	hCP51533	14	enzyme	1.2	3.5	ND
GNG2	Guanine nucleotide binding protein (G protein), gamma 2	hCG22671	hCT13769	hCP40131	14	Signaling	0.9	3.2	Yes

CDCA7	Cell-division cycle associated 7	hCG16803	hCT7844	hCP34351	2	Unknown (nuclear)	ND	ND	Yes
ENC1	Ectodermal-neural cortex	hCG37104	hCT1957908/hCT40185	hCP48121/hCP1766097	5	Unknown	4.1	2.6	Yes
STXBP6	Syntaxin binding protein 6 (amisyn)	hCG40185	hCT1813210/hCT31439	hCP1768217/hCP50010	14	Unknown	ND	ND	ND
Image ID 1048671	Unknown	hCG1645335	hCT1645462	hCP1634454	X	Unknown	ND	ND	Yes
Image ID 1871074	Unknown	Unknown	Unknown	Unknown	2	Unknown	ND	ND	Yes
Image ID 376697	LOC285705 (PROVISIONAL)	hCG27486	hCT18626	hCP43057	5	Unknown	ND	ND	Yes
Image ID 294873	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	ND	ND	ND
Image ID 940994	Unknown	Unknown	Unknown	Unknown	11	Unknown	ND	ND	Yes
Image ID 742837	LOC286459 (PROVISIONAL)	hCG39573	hCT30826	hCP49367	X	Unknown	ND	ND	ND
Image ID 753028	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	ND	ND	ND
TM4SF9	Tetraspan 5	hCG37727	hCT28961	hCP48431	4	Membrane	ND	ND	ND
Image ID 294133	FLJ12747 (PROVISIONAL)	hCG40978	hCT32246	hCP50788	22	Unknown	ND	ND	ND
Image ID 489594	FLJ11565 (PROVISIONAL)	hCG1811066	hCT1951376	hCP1751927	X	Unknown	ND	ND	ND

A method for the development of the therapeutic compounds according to the invention comprises the steps of:

5 a) identification of genes regulated by TCF/ β -catenin in colon carcinoma cells, in particular by using microarray technologies;

b) validation of one or more of the identified genes as potential target gene(s) for the therapeutic compound by one or more of the following methods:

- 10 - confirmation of the identified gene by Northern Blot analysis in colon carcinoma cell-lines;
- determination of the expression profile of the identified gene in human colorectal tumors and normal tissue;
- 15 - determination of the functional importance of the identified target genes for colorectal cancer;

c) production of the expression product of the target gene; and

d) use of the expression product of the target gene for the production or design of a therapeutic compound.

25 Once the target gene is validated and the expression product of the gene (the expressed protein) is produced there are various ways for developing a therapeutic compound for treating colorectal cancer.

In colorectal carcinoma cells the TCF/ β -catenin regulated target genes identified according to the invention are over-expressed upon constitutive TCF/ β -catenin activity. The compounds of the invention should thus neutralize the biological activity of the proteins expressed by the target gene in order to reverse the

carcinoma phenotype.

A known way of neutralizing proteins is by means of antibodies. The invention according to a first aspect thereof thus relates to antibodies directed against the expression products of the target genes listed in Table 1 for use in the treatment of colorectal cancer. The production and evaluation of antibodies and their derivatives, such as scFv, Fab, chimeric, bifunctional and other antibody-derived molecules are well within the reach of the skilled person. Therapeutic antibodies are in particular useful against target gene expression products located on the cellular membrane.

A second aspect of the invention relates to so-called "small molecules" interfering with the biological activity of the protein expressed by the target gene for use in the treatment of colorectal cancer. Small molecules are usually chemical entities that are developed on the basis of structure-function analysis of the protein with which they should interfere. Such analysis may involve determination of the crystal structure of the target protein. Based on the information thus obtained libraries of compounds can be screened or compounds may be designed and synthesized using medicinal and/or combinatorial chemistry. Alternatively, high throughput screening can be used to generate useful drug lead compounds as well. After identification of a lead compound, this compound is screened for inhibition of target protein function using in vitro and/or cell-based assays. After optimization of the lead compound with respect to its structure, toxicity profile and inhibition capability, its efficacy as colon cancer therapeutic is tested in vivo using animal models (e.g. Xenograft, APC^{min} mouse).

According to a third aspect of the invention

antisense molecules are provided. Antisense drugs are complementary strands of small segments of mRNA. To create antisense drugs, nucleotides are linked together in short chains called oligonucleotides. Each antisense drug binds to a specific sequence of nucleotides in its mRNA target to inhibit production of the protein encoded by the target mRNA. By acting at this earlier stage in the disease-causing process to prevent the production of a disease-causing protein, antisense drugs have the potential to provide greater therapeutic benefit than traditional drugs which do not act until the disease-causing protein has already been produced. The invention relates to antisense molecules directed against the target genes listed in Table 1.

A further aspect of the invention relates to RNA interference (RNAi) molecules. RNAi refers to the introduction of homologous double stranded RNA to specifically target a gene's product, resulting in a null or hypomorphic phenotype. RNA interference (RNAi) requires an initiation step and an effector step. In the first step, input double-stranded (ds) RNA is processed into 21-23-nucleotide "guide sequences". These may be single- or double-stranded. The guide RNAs are incorporated into a nuclease complex, called the RNA-induced silencing complex (RISC), which acts in the second effector step to destroy mRNAs that are recognized by the guide RNAs through base-pairing interactions. RNAi molecules are thus double stranded RNAs (dsRNAs) that are very potent in silencing the expression of the target gene. Potentially, a single dsRNA molecule could mark hundreds of mRNAs for destruction.

The invention relates further to gene therapy, in which the target genes are used for the design of dominant-

negative genes which inhibit the function of the corresponding target gene following their specific expression in a cancer cell. Alternatively, RNAi approaches can be used gene therapeutically, for example by

5 introducing a dsRNA producing sequence into a cancer cell.

The invention further relates to pharmaceutical compositions for treating cancers in which TCF/ β -catenin signalling is deregulated, in particular colorectal cancer and melanomas, said compositions comprising a suitable
10 excipient, carrier and/or diluent, and one or more inhibitors of the proteins expressed by the TCF/ β -catenin target genes, or inhibitors of the mRNAs of the target genes.

The invention also provides diagnostic methods
15 for diagnosing cancer, in particular colorectal cancer and melanomas, comprising histological examination of tissue specimens, using specific antibodies directed against TCF/ β -catenin target gene products and/or in situ hybridisation analysis of TCF/ β -catenin target gene
20 expression using specific RNA probes directed against TCF/ β -catenin target genes.

The present invention will be further illustrated by the following, non-limiting, Examples. In the Examples reference is made to the following figures:

25 **Figure 1.** TCF/ β -catenin driven transactivation is abrogated upon induction of dominant-negative TCF (dnTCF).

(A) Inducible dnTCF4 expression in the CRC line Ls174T. Cells were stained for dnTCF4, 24 hours after induction with doxycycline. DnTCF4 is highly expressed in
30 the nucleus.

(B) dnTCF4 protein is induced as early as 4 hours after induction with doxycycline as analysed by western

blot.

(C) Both dnTCF1 and dnTCF4 abrogate β -catenin/TCF driven transcription in the β -catenin-mutant Ls174T as well as in the APC-mutant DLD1 cells. Activity of the TCF-reporter TOPFlash (purple bars) and control FOPFlash (green bars) after 24 hours with or without doxycycline (dox) is shown. Parental cell lines expressing the Tet-repressor alone are used as controls.

Figure 2. Northern blot analysis of genes regulated by β -catenin/TCF activity.

(A) Representative examples of several Tcf/ β -catenin target genes in Ls174T and DLD-1 colon carcinoma cells. The indicated mRNAs were down-regulated following 24 hours of doxycycline-induced expression of dominant-negative Tcf (dnTCF). The bottom panel shows the 28S ribosomal RNA as a loading control. (Dox: Doxycycline; CON: RNA from control cells lacking dominant-negative Tcf expression).

(B) Additional examples of genes whose expression is dependent upon active TCF/ β -catenin signalling in LS174T colon cancer cell-lines. The indicated mRNAs were down-regulated following 24 hours of doxycycline induced expression of dominant-negative TCF.

Figure 3. Expression of dnTCF induces G1-cell cycle arrest in colon carcinoma cells.

(A) Ls174T and DLD1 show a dramatic reduction in S phase cells upon dnTCF expression. The scatter profile of cells in G1 (blue), S (green) and G2/M (red) after 20 hours with or without doxycycline is shown. Numbers refer

to the percentage of cells in S phase for each cell line analyzed. The results are representative of several independent experiments.

(B) Proliferation was halted in Ls174T and DLD1 transfectants. This was visualized by crystal violet staining of cell cultures after 5 days of dnTCF expression.

Figure 4. (A-C) The expression of nuclear β -catenin (A, black arrowheads) perfectly correlates with that of EPHB2 tyrosine kinase receptor (B, black arrowheads) in aberrant crypt foci (ACF). Stainings were performed on serial sections of early human lesions. The dashed lines delimit the same ACF in both stainings. EPHB2 is expressed at the bottom of the crypts (C, white arrowheads).

Figure 5. Model for the role of β -catenin/TCF in the early stages of intestinal tumorigenesis.

(A) Schematic representation of a colon crypt and proposed model for polyp formation. At the bottom third of the crypt, the progenitor proliferating cells accumulate nuclear β -catenin. Consequently they express β -catenin/TCF target genes. An uncharacterised source of WNT factors likely resides in the mesenchymal cells surrounding the bottom of the crypt, depicted in red. As the cells reach the mid-crypt region, β -catenin/TCF activity is downregulated and this results in cell cycle arrest and differentiation. Cells undergoing mutation in APC or β -catenin become independent of the physiological signals controlling β -catenin/TCF activity. As a consequence, they continue to behave as crypt progenitor cells in the surface epithelium giving rise to ACFs.

(B) CD44, a β -catenin/TCF target, exemplifies this model. It is expressed in the normal proliferative

compartment at the bottom of the crypts (white arrowheads) and also in the early lesions arising at the surface epithelium (black arrowheads).

Figure 6. Expression of β -catenin/TCF target genes in normal colon and colorectal polyps.

(A-F) Immunohistochemical analysis of the expression of Bmp4 (A and B), cMyb, (C and D) and Encl (E and F) in normal mouse colon (A, C and E) or colorectal polyps from *min* mice (B, D and F). Target genes are highly expressed at the bottom of the normal crypts (white arrowheads) and in colorectal polyps arising at the surface epithelium (black arrowheads).

Figure 7. Expression of EPHB3 and EPHB4 in normal colon and colorectal polyps. (D,F,G) EPHB3 and EPHB4 are expressed in the crypts of normal mouse colon (White arrowheads, D,E respectively) and are over-expressed in intestinal polyps of APC^{min} mice (Black arrowheads, F,G respectively). EPHB3 expression is absent in TCF-4 knock-out mice deficient in TCF-4/ β -catenin signalling in the small intestine(H).

Figure 8. (A) Semi-quantitative RT-PCR analysis of selected TCF target gene expression levels in human cancer cell-lines. **(B)** Summary table of estimated TCF target gene expression levels in various cancer cell-lines (- undetectable expression, + low level expression, ++ moderate expression, +++ high-level expression, ++++ very high level expression, ND not determined, L 100bp DNA ladder).

Figure 9. Quantification of selected TCF target gene expression levels in human cancer cell-lines.

(A) Endogenous GPX2 mRNA levels in a panel of 20 human cancer cell lines of varying origin. Q-PCR analysis

of endogenous GPX2 expression levels reveals high-level expression in cancer cell-lines of varying origin, with particularly high levels evident in colon carcinoma cell-lines LS174T, HT29, SW480, the lung cancer cell-line A549 and the breast cancer cell-line MDAMB351. Numbers above the figure correspond to very high GPX2 mRNA values not shown to scale.

(B) Endogenous EPHB2 mRNA levels in a panel of 20 human cancer cell lines of varying origin. Q-PCR analysis of endogenous GPX2 expression levels reveals high-level expression in cancer cell-lines of varying origin, with particularly high levels evident in colon carcinoma cell-lines LS174T, DLD-1 and the prostate cancer cell-line PC3. Numbers above the figure correspond to very high EPHB2 mRNA values not shown to scale.

(C) Endogenous RGMR mRNA levels in a panel of 20 human cancer cell lines of varying origin. Q-PCR analysis of endogenous RGMR expression levels reveals high-level expression in selected colon, lung and prostate cancer cell-lines, with highest levels evident in the colon cancer cell-line LS174T.

(D) Endogenous Tspan5 mRNA levels in a panel of 20 human cancer cell lines of varying origin. Q-PCR analysis of endogenous Tspan5 expression levels reveals high-level expression in selected colon, lung and prostate cancer cell-lines, with highest levels evident in the colon cancer cell-line HCT116.

Figure 10. (A) Schematic representation of the CD44 gene. Open boxes indicate exons that can be alternatively spliced. TM: transmembrane region.

(B, C) Schematic representation of the CD44 protein with localizations of the epitopes that are recognized by the anti-human monoclonal antibodies VFF18

and Hermes-3 and the anti-mouse antibodies PGP-1, 10D, and 9A4. v1 to v10, domains encoded by variant exons.

Figure 11. Exon/Intron organization of human CD44. Exons encode the constant part of the extracellular domain. Exons 6-15 correspond to variant exons 1-10 respectively and encode for the extracellular domain (Variant exon 1 is not expressed in humans). Exons 16 and 17 are constant exons, which together with part of exon 5 encode the membrane proximal region of the extracellular domain. Exon 18 encodes the hydrophobic transmembrane region and exons 19 and 20 encode the cytoplasmic domain. Exons 19 and 20 are also subject to alternative splicing, generating either long or short cytoplasmic domains.

Figure 12. CD44 expression requires TCF/ β -catenin signalling.

(A) Inhibition of TCF/ β -catenin signalling using dnTCF results in loss of CD44 expression on the cell-surface of colon cancer cells (Pan-CD44Mab used).

(B) CD44 expression is lost in intestinal crypts following deletion of TCF-4 in mice.

Figure 13. CD44 is over-expressed in early colorectal polyps in comparison to normal colon. Stainings were generated using a Pan-CD44 antibody.

Figure 14. Schematic representation of GPR49 which belongs to the G protein-coupled receptor (GPCR) superfamily with a large seven-transmembrane (TM).

Figure 15. Lineup of RGM and RGMR Protein Sequences.

Figure 16. Schematic representation of Tspan5, which comprises 4 transmembrane domains and two large extracellular loops.

Figure 17. cDNA and protein sequence of CD44: A. cDNA Sequence (hCT1951772/NM_000610), B. Protein

sequence (hCP1753227/ NP_000601).

Figure 18. (A) cDNA sequence: X55150 (alternative splice variant CD44E), (B). Protein sequence: S13530 (alternative splice variant CD44E).

5 **Figure 19.** CD44 alternative splice variants containing any combination of the following variable exons:

A. Variable exon 2 (L05411), B. Variable exon 3 (L05412),
C. Variable exon 4 (L05413), D. Variable exon 5 (L05414),
E. Variable exon 6 (L05415), F. Variable exon 7 (L05416),
10 G. Variable exon 8 (L05417), H. Variable exon 9 (L05418),
I. Variable exon 10 (L05419).

Figure 20. cDNA and protein sequence of GPR49: A. cDNA Sequence: hCT14878, B. Protein Sequence: hCP42243

15 **Figure 21.** cDNA and protein sequence of EPHB4: A. cDNA Sequence: hCT11528/ NM_004444, B. Protein Sequence, hCP38155/ NP_004435.

Figure 22. cDNA and protein sequence of GPX2: A. cDNA sequence: X68314, B. Protein Sequence:
20 CAA48394.

Figure 23. cDNA and protein sequence of hCG27486 (RMGR): A. cDNA sequence: hCT18626, B. Protein Sequence: hCP43057.

Figure 24. cDNA and protein sequence of Tspan5:
25 A. cDNA sequence: NM_005723, B. Protein sequence: NP_005714.

EXAMPLES

EXAMPLE 1

Identification of target genes

5

MATERIAL AND METHODS

Cell culture and transfections

Cells were grown in RPMI supplemented with 10% FCS and antibiotics. T-REx system (Invitrogen) was used according to manufacturer's instructions to generate inducible dnTCF or p21^{CIP1/WAF1} inducible CRC cell lines. In short, 10⁷ cells were transfected by electroporation with 20 µg FspI linearized pcDNA₆TR . After 3 weeks of selection, blasticidin (10 µg/ml) resistant colonies were expanded and transfected with pcDNA₄TO-Luciferase. From each cell line, two clones showing the strongest induction were chosen. These were subsequently transfected with 20 µg PvuI linearized dnTCF1 or dnTCF4 in pcDNA₄TO. After selection on Zeocin (500µg/ml), resistant colonies were tested for dnTCF induction by immunocytochemical staining after addition of doxycycline and selected for further studies.

Cell cycle analysis

25 3 x 10⁶ (Ls174T) or 10⁶ (DLD1) cells were seeded in 9 cm dishes and doxycycline was added (1µg/ml). After 20 hrs, BrdU (Roche) was added for 20 min. Cells were then collected and fixed in ethanol 70%. Nuclei were isolated, incubated with α-BrdU-FITC (BD) and cell cycle profiles were determined by FACS analysis. Crystal violet staining on methanol fixed cells was performed on cells after 5 days in culture with or without the addition of doxycycline.

RNA isolation and northern analysis

RNA was isolated using Trizol (Gibco). Northern blots were performed according to standard procedures .

- 5 Probes were obtained by appropriate restriction enzyme digestion of corresponding IMAGE clones (IMAGE consortium) spotted in the array.

Immunohistochemistry

- 10 The antibodies used in this study were obtained from the following sources: EPHB2, EPHB3 and EPHB4 from R&D systems; BMP4 from Novacastra; ENC1 from Pharmingen; MYB from Santa Cruz Biotechnology; p21^{CIP1/WAF1} from Pharmingen; carbonic anhydrase II from Rockland; β -catenin from
- 15 Transduction Laboratories; TCF1 and TCF4 antibodies were described elsewhere. Immunostainings were performed according to standard procedures. Briefly, sections were pretreated with peroxidase blocking buffer (100 mM Na-phosphate pH 5.8, 30 mM NaN₃, 0.2% H₂O₂) for 20 minutes at
- 20 room temperature after dewaxing and hydration. Antigen retrieval was performed by boiling samples in 10 mM Na-citrate buffer pH 6.0, for 20 minutes. For β -catenin stainings, samples were boiled for 45 minutes in 40 mM Tris pH 8.0 containing 1 mM EDTA. Incubation of antibodies was
- 25 performed in 1% BSA in PBS 1 hour at room temperature. In all cases, the Envision+ kit (DAKO) was used as a secondary reagent. Stainings were developed using DAB (brown precipitate). Slides were then counterstained with hematoxylin and mounted.

Probe preparation and microarray procedures

mRNA was extracted from cells using the Fasttrack 2.0 procedure (Invitrogen Inc.) following the manufacturer's directions. Fluorescent labeled cDNA was prepared from 1 μ g of polyA mRNA by oligo dT-primed polymerization using Superscript II reverse transcriptase in the presence of either Cy3- or Cy5- labeled dCTP as described (website: <http://cmgm.stanford.edu/pbrown/protocols.html>). The appropriate Cy3- and Cy5- labeled probes were pooled and hybridized to microarrays in a volume of 25 μ l under a 22x14 mm glass coverslip for 16 hr. at 65°C and washed at a stringency of 0.2XSSC. The microarray contains 24,000 DNA spots representing approximately 10,000 known full-length cDNAs and 14,000 ESTs of clones made available by Research Genetics, which are listed in the supplementary information.

Fluorescent images of hybridized microarrays were obtained using a genepix 4000 microarray scanner (Axon Instruments, Inc). Images were analyzed with scanalyze (M.Eisen; <http://www.microarrays.org/software>) or with genepix 3.0. Fluorescence ratios were stored in a custom database. Fluorescent ratios were calibrated independently for each array by applying a single scaling factor to all fluorescent ratios from each array; this scaling factor was computed so that the median fluorescence ratio of the measured spots on each array was 1.0. Genes represented by good-quality spots for which the fluorescent intensity in each channel was greater than 1.5 times the local background were selected.

RESULTS

Generation and characterization of inducible dnTCF cell lines

5 To determine the role of β -catenin/TCF complexes in established CRC cells, cell lines were constructed carrying doxycycline-inducible expression plasmids encoding N-terminally truncated versions of TCF factors. Such dominant-negative TCF (dnTCF) proteins do not bind β -
10 catenin and therefore act as potent inhibitors of the endogenous β -catenin/TCF complexes present in CRC. As the recipient cell line, the CRC cell line Ls174T, which expresses mutant β -catenin protein, yet is diploid and carries wild-type alleles of *p53* and *APC* was initially
15 chosen. Multiple clones were isolated and tested for inducibility of dnTCF4 expression.

 Strong nuclear dnTCF4 staining was observed after doxycycline (Dox) induction of positive transfectants (Figure 1A). Accumulation of the induced protein could be
20 detected as early as 4 hours after the addition of doxycycline (Figure 1B). CRC cell lines such as Ls174T that carry WNT pathway mutations constitutively activate TCF reporters (pTOPFlash). Several clones were selected in which the inducible expression of dnTCF4 completely
25 abrogated this constitutive pTOPFlash activity (Figure 1C). Induction of dnTCF4 in such clones imposed a robust cell cycle block (see below), but did not result in the onset of apoptosis.

30 The genetic program under the transcriptional control of β -catenin/TCF activity in CRC cells

 The spectrum of target genes controlled by β -catenin/TCF in CRC cells was expected to hold the key to

understanding the primary transformation of intestinal cells. By DNA array analysis, it was determined which genes were specifically affected in their expression upon the induction of dnTCF4. mRNA was isolated at 11 hours and 23
5 hours after the initiation of the experiment with or without the addition of doxycycline. cDNA prepared from the uninduced samples was labeled with Cy3, while the induced cDNA samples were labeled with Cy5. At each time point, the uninduced and induced cDNA samples were mixed and
10 hybridized in duplicate to a DNA array consisting of approximately 24,000 cDNA spots representing known genes or EST clusters. Fluorescent images were analysed as detailed in the experimental procedures.

A single criterium was applied to the array data
15 set: i.e. a decrease of at least 2.5 fold in both measurements at the 23 hour time point. This defined a small set of 35 entries that were downregulated when β -catenin/TCF activity was abrogated in Ls174T cells expressing dnTCF-4 (listed in Table 1).

20 For a number of downstream genes defined in the Ls174T cells expressing dnTCF4, Northern blot analysis was performed before and after induction of dnTCF4. This invariably confirmed the DNA array data (Table I and Figure 2A/2B). The down-regulation of the reported TCF4 target
25 gene *c-MYC* did not meet the 2.5-fold selection criterion, decreasing by an average 1.8-fold. However, its relatively modest, but consistent down-regulation was confirmed by northern blot (Figure 2A).

To further investigate the effects of β -
30 catenin/TCF inhibition, Ls174T cells expressing dnTCF1, the natural dominant-negative isoform of TCF1 expressed in the intestinal epithelium, were constructed. Likewise, DLD-1

cells, a CRC cell line with wild type β -catenin but mutated APC and p53, was engineered to express inducible dnTCF1 or dnTCF4.

Again, doxycycline-induced expression of dnTCFs
5 in all cell lines resulted in the abrogation of pTOPflash activity (Figure 1C) and cell cycle arrest (see below). Northern blot analysis was performed in the above dnTCF expressing cell lines. Almost invariably, the target genes listed in Table I were also strongly downregulated by
10 dnTCF1 in Ls174T (Figure 2A). In addition, the DLD-1 cells showed a similar pattern of target gene expression upon inhibition of β -catenin/TCF activity by dnTCF1 or dnTCF4 (Figure 2A).

15 Inhibition of β -catenin/TCF activity leads to cell cycle arrest and differentiation in CRC cells.

The induction of dnTCF4 or dnTCF1 in both Ls174T and DLD1 cell lines had a dramatic effect on cell cycling. Within 20 hours, a robust G1 arrest was induced (Figure
20 3A). Accordingly, cell proliferation was halted upon doxycycline induction of dnTCFs as visualized by crystal violet staining of cell cultures induced for 5 days (Figure 3B).

25 The genetic program controlled by β -catenin/TCF in CRC cells is physiologically active in colonic epithelium

In order to validate the β -catenin/TCF target genes described in this example, immunohistochemical
30 analyses of those entries for which antibodies were available were performed on early intestinal neoplastic lesions. In Figure 4, a representative example of this

analysis is shown. As expected, a strict correlation between the accumulation of nuclear β -catenin (Figure 4A) and the expression of EPHB2 (Figure 4B) was observed in early colorectal lesions. Many other downregulated genes listed in Table I were overexpressed in early intestinal polyps from *Min* mice or in aberrant crypt foci (ACF) from FAP patients (Figure 5, Figure 6, Figure 7).

More strikingly, it was found that EPHB2 was not only expressed in polyps, but also in cells within the proliferative compartment at the bottom of normal colon crypts (Figure 4C). This pattern was invariably confirmed for all target genes tested by immunohistochemistry. These included *MYB*, *BMP4*, *ENC1*, (Figure 6), *EPHB3* (Figure 7), and *CD44* (Figure 5B).

Thus, the observed gene expression changes in CRC cells recapitulated the physiological differentiation of crypt progenitor cells during their migration towards the luminal surface of the intestine.

DISCUSSION

The data presented here provide a view of the genetic program driven by β -catenin/TCF activity in CRC cells. The expression of a surprisingly limited set of genes is dependent on the presence of active β -catenin/TCF complexes.

A hallmark of cancer is deregulated proliferation. Abrogation of β -catenin/TCF activity in all CRC cell lines tested induced a robust arrest in the G1 phase of the cell cycle, demonstrating that the activity of the β -catenin/TCF complex represents the major force driving cell proliferation in intestinal cells.

β -Catenin and TCF modulate cell cycle control by activating genes that promote cell cycling (e.g. c-myc), but also by repressing cell cycle inhibitors (p21^{CIP1/WAF1}; results not shown). β -catenin/TCF represents the main upstream regulator of the cell cycle machinery in epithelial intestinal cells.

In conclusion, the above observations demonstrate that TCF constitutes the dominant switch between the proliferating progenitor and the differentiated intestinal cell. This is recapitulated in the CRC cells used in this study, despite the presence of multiple additional mutations in these cells. This example validates that the genetic program controlled by TCF/ β -catenin signalling can be used as the basis for the development of a therapeutic strategy to revert the transformed phenotype in colorectal cancer.

EXAMPLE 2

Development of drugs for the treatment of colorectal cancer on the basis of the target genes of Example 1

Identification and validation of the target genes

Example 1 demonstrates the identification of target genes represented on cDNA/oligonucleotide microarrays which are regulated by TCF/ β -catenin transcription factor complexes. Subsequently, the regulated expression of target genes in colon cancer cell-lines is confirmed via Northern blot analysis using gene specific probes as described in Example 1.

In order to confirm that the expression of the target genes that were found in Example 1 are linked to the TCF/ β -catenin complex, target gene expression is also

evaluated in tissues known to have active TCF/ β -catenin complexes (for example, intestinal epithelium and colorectal polyps) using gene-specific antibodies, in situ hybridization with gene-specific probes and/or RT-PCR with
5 gene-specific primers.

After that, the expression profile of the target gene in human/mouse cell-lines and tissues is determined via Northern blot analysis and/or RT-PCR. This is done because ubiquitous expression of the target gene may be
10 indicative of possible side-effects of therapeutics designed to block the target gene's function in vivo.

Obtaining the complete gene

The identification of the target genes on a
15 microarray does not identify the complete gene. The next step in the development of a therapeutic compound is therefore the generation of full-length clones for the EST sequences which are shown to be regulated via TCF/ β -catenin in the colon carcinoma cell-lines. This is achieved by
20 searching databases for full-length EST clones and/or techniques such as RT-PCR, RACE and hybridization screening of cDNA libraries.

25 Identification of binding sites within the target genes

Putative TCF binding sites [(A/T) (A/T) CAA (A/T) GG] within target gene promoters are identified according to Van de Wetering et al. (Identification and cloning of TCF-1, a T-lymphocyte-specific transcription factor containing
30 a sequence-specific HMG box; EMBO J. 11: 3039-3044, 1991). Enhancers are identified using web-based prediction programs such as Genomatix (www.genomatix.gsf.de/promoterinspector). This provides an

indication that a gene is regulated via direct binding of TCF/ β -catenin complexes. However, many binding sites will not be identified due to the vast tracts of genomic DNA containing the target gene which may harbor distant enhancers. Testing of the functionality of putative TCF binding sites in target genes is then performed via mutational analysis. Promoter regions of target genes containing the original or mutated putative TCF binding sites are cloned upstream of TK-Luciferase reporter genes cassettes and analysed for their ability to drive expression of the reporter gene in the presence of TCF/ β -catenin complexes in cultured cell-lines, such as described in Tetsu and McCormick, (1999) (β -catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 398: 422-426)). A correlation between mutation of a TCF binding site and loss of reporter gene expression indicates that direct binding of TCF/ β -catenin is contributing to target gene expression.

Determination of the ability of ectopic target gene expression to overcome defects in the growth of colon carcinoma cells caused by blocking TCF/ β -catenin signalling is performed as described in Example 1 to establish whether expression of a single target gene is sufficient to overcome the block in cell-cycle and differentiation of the colon cancer cell.

Confirmation of involvement of target genes in colon cancer

Subsequently it is important to establish the contribution of specific target genes to colon cancer. The techniques used to do this are dominant-negative approaches, i.e. expression of target genes carrying deletions/mutations which suppress the function of their

endogenous counterparts in colon cancer cell-lines;
antisense/RNAi approaches, i.e. introduction of double-
stranded RNA oligonucleotides designed to block expression
of a specific target gene into colon cancer cell-lines, as
5 described by Elbashir et al. (Duplexes of 21-nucleotide
RNAs mediate RNA interference in cultured mammalian cells.
Nature 411: 494-498, 2001) and genetic approaches, such as
generation of mice deficient for target gene expression in
intestinal tissues using a combination of standard loxP
10 knockout technologies and intestine-specific Cre mouse
strains, or generation of transgenic mice expressing
dominant-negative target genes. These mice strains are
crossed with APC^{min} mice to determine whether loss of target
gene function in vivo has any adverse effect on colorectal
15 polyp formation (as for example described by Oshima et al.
(Suppression of Intestinal Polyposis in APC^{D716} Knockout
mice by inhibition of cyclooxygenase 2 (COX-2) Cell 87:
803-809, 1996).

Using these approaches it is determined whether
20 loss of function of a specific target gene has any adverse
effect on colon cancer cell-lines and/or on polyp formation
in vivo and thus insight is gained into whether
therapeutics designed to specifically inhibit the function
of these target genes are likely to be effective in
25 combating colon cancer in humans.

Furthermore, the genetic programs affected by
inhibition of target gene function in colon carcinoma cells
are evaluated using microarrays. Thus, the function of the
target gene in colon carcinoma cells is established and
30 valuable information regarding the possible side-effects
that inhibition of this gene function may have on genetic
programs required for normal cell function is provided. By
definition, many of the validated TCF/ β -catenin target

genes will be more highly expressed on colon carcinoma tissues than healthy tissues and some encode cancer-specific proteins, making these excellent targets against which to develop colon cancer therapeutics.

5

Identification or development of candidate compounds

Antibodies

Validated target genes which express membrane-bound proteins are then selected as targets for conventional antibody-based therapies, for example according to Schwartzberg (Clinical experience with edrecolomoab: a monoclonal antibody therapy for colorectal carcinoma. Crit. Rev. Oncol. Hematol. 40: 17-24, 2001).

15

Small molecules

Validated intracellular and membrane-expressed target proteins are furthermore selected as targets for developing small molecule compound-based therapies. For this their crystal structures are determined, either from published information available from web-based databases (NCBI) or using protein production and crystallization facilities. Structure analysis is performed with the computer programs SPOCK (Christopher J. (1998). SPOCK, The structural properties observation and calculation kit, 1998), GRASP (Nicholls et al., (1991) Structure, Function and Genetics 11:281-283) and SWISS PDB Viewer (Guez and Peitsch (1997) SWISS-MODEL and the Swiss-Pdb Viewer: An environment for comparative protein modeling) or others.

25

In addition to the rational development of novel small molecules, high capacity screening of existing small molecule compound libraries generate lead compounds which become inhibitors of validated target proteins encoding

enzymes such as protein kinases. Highly active inhibitors are co-crystallized with the enzyme and computer programs such as GOLD (Distributed via Cambridge Crystallographic Data Centre; Jones et al., (1995) J. Mol. Biol 245: 43-53) and CERIUS2/LUDI (Bohm (1992) The computer program Ludi: A new method for the de novo design of enzyme inhibitors. J. Comp. Aided Molec. Design 6:61-78) are used for structure-based design of improved inhibitors.

10 Antisense molecules

These can be either antisense RNA or antisense oligodeoxynucleotides (antisense ODNs), and can be prepared synthetically or by means of recombinant DNA techniques. Both methods are well within the reach of the person skilled in the art. ODNs are smaller than complete antisense RNAs and have therefore the advantage that they can more easily enter the target cell. In order to avoid their digestion by DNase, ODNs, but also antisense RNAs, are chemically modified. For targeting to the desired target cells the molecules are linked to ligands of receptors found on the target cells or to antibodies directed against molecules on the surface of the target cells.

25 RNAi molecules

Double-stranded RNA corresponding to a particular gene is a powerful suppressant of that gene. The ability of dsRNA to suppress the expression of a gene corresponding to its own sequence is also called post-transcriptional gene silencing or PTGS. The only RNA molecules normally found in the cytoplasm of a cell are molecules of single-stranded mRNA. If the cell finds molecules of double-stranded RNA, dsRNA, it uses an enzyme to cut them into fragments

containing 21-25 base pairs (about 2 turns of a double helix). The two strands of each fragment then separate enough to expose the antisense strand so that it can bind to the complementary sense sequence on a molecule of mRNA.

5 This triggers cutting the mRNA in that region thus destroying its ability to be translated into a polypeptide. Introducing dsRNA corresponding to a particular gene will knock out the cell's own expression of that gene. This can be done in particular tissues at a chosen time. A possible

10 disadvantage of simply introducing dsRNA fragments into a cell is that gene expression is only temporarily reduced. However, introducing into the cells a DNA vector that can continuously synthesize a dsRNA corresponding to the gene to be suppressed can provide a more permanent solution.

15 RNAi molecules are prepared by methods well known to the person skilled in the art.

Other compounds

To predict the location of critical contact sites

20 for cofactors, ligands or other molecules contributing to the function of target proteins, use is made of computer-based modeling with the programs mentioned above. Confirmation of essential contact sites in target proteins is performed by mutational analysis and subsequent

25 identification of hydrophobic pockets located on the protein surface in the vicinity of these contact sites.

Computer modeling of "virtual" public compound libraries for binding to these hydrophobic pockets and testing of "best fit" compounds in in vitro (ELISA) and in

30 vivo (cell-based) assays for inhibition of target protein function will allow determination of a structure-activity relationship for compound classes.

In addition, de novo compound libraries are

generated based on the information derived from the computer modeling described above using a combinatorial chemistry approach.

5 Evaluation of candidate compounds

Candidate compound are evaluated for cellular toxicity via commercially available service, such as MDS Pharma Services, USA. The evaluation of candidate compound efficacy in reducing polyp formation in APC^{min} mice
10 according to Su et al., (1992) Multiple intestinal neoplasia caused by a mutation in the murine homologue of the APC gene. Science 256: 668-670. The candidate compounds are also tested in other models predictive for colorectal cancer (e.g. Xenograft).

15

EXAMPLE 3

Quantitative PCR (Q-PCR)

A panel of 20 different cancer cell lines derived
20 from lung (HOP-62, A549, EKVX), colon (DLD-1, Ls174T, HT29, HCT-116, SW480), breast (MDA-MB-435s, MCF7, T47D, MDA-MB-361, MDA-MB-231, MDA-MB-468) prostate (DU145, PC3), ovarian (IGROV-1, OVCAR-4) and melanoma (M14, SK-MEL-5) were lysed and total RNA was extracted using Trizol[™] reagent
25 according to manufacturers' instructions. One microgram of RNA was reverse transcribed to generate the corresponding cDNA, which was used as the template for Q-PCR. The reverse transcription step was performed in 96-well plates using the TaqMan reverse transcription kit (Applied Biosystems)
30 according to the manufacturer's recommendations. The cDNA was quantified by the SyBR green method using a SyBR Green PCR master Mix kit (Applied Biosystems) according to the manufacturer's recommendations. For each reaction, 8 ng of

cDNA was used as a template and 300 nM of specific forward and reverse oligonucleotides added. Duplicate experiments were carried out using an Applied Biosystems 7000 SDS.

Values were normalized according to the β -glucuronidase

5 (GUS) gene, which was measured as internal control.

Oligonucleotides were designed using the Primer Express software (Applied Biosystems). These oligonucleotides were validated by Q-PCR experiments to obtain a quantitative measurement (quantification of serially diluted cDNA and
10 determination of PCR efficiency). The sequences of the oligonucleotides for internal control were as follows:

GUS: forward 5'-CCCGCGGTCGTGATGT-3'
reverse 5'-TGAGCGATCACCATCTTCAAGT-3'

15

The sequences of the oligonucleotides used to probe the cDNA of the selected genes were:

EPHB2: forward 5'- TCTTCCTCATTTGCTGTGGTTGT-3' (SEQ ID No. 13)

20 reverse 5'- TGTTGCAGCTTGTCCGTGTAC-3' (SEQ ID No. 14)

GPX2: forward 5'- CAGGGCCGTGCTGATTG-3' (SEQ ID No. 15)

reverse 5'- CTCGTTGAGCTGGGTGAAGTC-3' (SEQ ID No. 16)

RGMR: forward 5'- AGGAACGCTGGCACATTTTC-3' (SEQ ID No. 17)

reverse 5'- TGAGTCCTAGACTGACAGACAAATCA-3' (SEQ ID No.

25 18)

Tspan5: forward 5'- CTTCAATTGCACAGATTCCAATG-3' (SEQ ID No.

19)

reverse 5'- GGATCTTTAGTGCAGCAGGAGAA-3' (SEQ ID No.

20)

30

Semi-Quantitative RT-PCR

Total RNA was prepared from 18 different human cancer cell lines of various origin using Fenezol™

according to the manufacturer's instructions (ActiveMotif, Belgium). First-strand cDNA was prepared from 1ug of total RNA using oligo dt (18) primers and MMLV RNA'se H minus point mutant reverse transcriptase (Promega). 2ul of the
5 25ul total first-strand cDNA was used as template in hot-start PCR's to amplify cDNA fragments corresponding to specific regions of the specified target gene products using primers spanning exon-intron boundaries (see below). GAPDH cDNA fragments were amplified from each cDNA sample
10 as an internal control for cDNA quality.

Specific primer combinations used to amplify the target gene cDNA fragments:

15 **Tspan5:**

Forward 5' - GCGAATTTCGTGTCCGGGAAGCACTACAAG- 3' (SEQ ID No. 21)

Reverse 5' - GCGAATTCCGCCAGCTCGCCCTGACAGCTT -3' (SEQ ID No. 22)

20

RGMR

Forward 5' - CACGCAGGTGACTGCCAACAG -3' (SEQ ID No. 23)

Reverse 5' - CGTCATCGATGCGTTCCTCA - 3' (SEQ ID No. 24)
25

EPHB3

Forward 5' - GGGTAACATCTGAGTTGGCGTGGA -3' (SEQ ID No. 25)

Reverse 5' - CATCGCCGTTGCAGTAGAGCTTG - 3' (SEQ ID No. 26)
30

GPR49

Forward 5' - CCTCAGTATGAACAACATCAGTCAG - 3' (SEQ ID No. 27)

Reverse 5' - GCTGATGTGGTTAGCATCCAGAC - 3' (SEQ ID No. 28)

GPX2

Forward 5' - GCGAATTCGCTTTCATTGCCAAGTCCTTC - 3' (SEQ ID No. 29)

Reverse 5' - GCCTCGAGCTATATGGCAACTTTAAGGAG - 3' (SEQ ID No. 30)

EXAMPLE 4**CD44**

CD44 is a multistructural and multifunctional cell surface receptor involved in cell-cell and cell-matrix interactions, cell trafficking, presentation of chemokines and growth factors to travelling cells and transmission of growth signals. The extracellular matrix component (ECM) hyaluronic acid (HA) is the principal ligand for CD44, but other ligands include other ECM components and mucosal addressin, serglycin, osteopontin and the class II invariant chain Li. Multiple isoforms of CD44 exist (at least 20) as a result of alternative splicing and posttranslational modifications. The resulting spectrum of products range in size from 85-23-Kda. The standard form of CD44, which is the smallest isoform (CD44s/CD44H; 85Kda following glycosylation) is the most abundant. It is widely expressed, but is found at highest levels on haematopoietic cells. Figure 10 shows a schematic representation of the CD44 gene.

Variant forms of CD44 (CD44v), generated by

alternative splicing around variable exons (V1-V10) encoding extracellular domains are often more restricted in their expression pattern. Expression of these variant forms is often associated with activation of T-cells and some
5 studies indicate a correlation between CD44v expression and cell proliferation. Many cancer cell types, as well as their metastases, express high levels CD44 and in some cancers there appears to be selection for high level expression of particular variant forms of CD44. In colon
10 cancer, there is often selection for high level expression of CD44v6 and CD44v9 (CD44v6/9 refers to all CD44 products containing variant exon 6/9 and will include many CD44 variants composed of different combinations of constant and variant exons). In these colon tumours this high level CD44
15 expression is driven by constitutive activation of the TCF/ β -catenin signalling pathway.

The contribution of CD44 to carcinogenesis is currently the focus of many studies. Over-expression of CD44 in invasive colorectal cancers is associated with the
20 presence of metastases and with an unfavourable patient prognosis. Interaction between CD44 and HA is proposed to promote cell motility and sometimes tumour growth and metastasis. Disruption of CD44 in metastatic mammary cancer cells has also been shown to induce apoptosis, implying a
25 role for CD44 in counteracting programmed cell death. Other studies indicate that inhibition of CD44 function in colon cancers through administration of soluble CD44-Ig fusion proteins or CD44 antisense RNA can retard tumour growth. Targeting of CD44 variants differentially expressed on
30 colon cancer cells using specific antibodies may therefore be a valid approach towards development of a safe and effective therapy for colon cancer.

TCF target gene status was confirmed by Northern

blot as shown in figure 2B. Figure 12 demonstrates that CD44 expression requires TCF/ β -catenin signalling. Thus, inhibition of TCF/ β -catenin signalling using dnTCF resulted in a loss of CD44 expression on the cell-surface of colon cancer cells. Furthermore it was demonstrated that CD44 was lost in intestinal crypts following deletion of TCF-4 in mice. Using pan-CD44 antibody for staining it was demonstrated that CD44 is over-expressed in early colorectal polyps compared to normal colon (Figure 13).

CD44v6 and CD44v9 were previously demonstrated to be over-expressed in colon cancer (Wielenga et al., Am. J. Pathol., 1999, 154: 515-523). Other variant forms were also more highly expressed in colon tumours than in normal intestine.

Soluble CD44-Ig fusion proteins have been shown to inhibit tumour formation by preventing binding of CD44 to its ligand(s). Accordingly, monoclonal antibodies are generated. These antibodies are preferably prepared using the full-length CD44 cDNA (containing variant exons V2-V10) for the generations of immunogens, in order to maximize the likelihood of obtaining monoclonal antibodies against different CD44 variants. Purified His-tagged fusion proteins generated from CHO cells and NS0 cell CD44 transfectants may be used to immunizetransgenic mice expressing human immunoglobulins (such as Humab mice), which will generate human antibodies against specified antigens. Monoclonal antibodies specific for CD44 are selected by screening NS0 transfectants using FACS analysis. Functional assays are then performed to determine the effects on apoptosis and proliferation/differentiation of colon cancer cells in vitro and the efficacy of tumour inhibition in mouse xenograft models.

cDNA and protein sequences of CD44 and variants

which preferably are used according to the present invention are given in Figure 17-19.

EXAMPLE 4

5

GPR49

Proteins in the large seven-transmembrane (TM), G protein-coupled receptor (GPCR) superfamily are functionally diverse and include receptors ranging from the cAMP receptor in slime mold to mammalian neurotransmitters and glycoprotein hormone receptors. GPR49 is most closely related (35% homology at protein level) to a subgroup of GPCRs that have a large N-terminal extracellular domain containing leucine-rich repeats which are important for interaction with large glycoprotein hormones, which leads to cAMP production in target cells via activation of G-proteins. GPR49 contains many more leucine-rich repeats than other members of this family, indicating that it may interact with larger (glycol) protein ligands. Specific functions for GPR49 are currently unknown. Expression of GPR49 is highest in muscle, placenta, spinal cord and brain, but is present at lower levels in colon, small intestine, bone marrow and adrenal gland (Hau et al. Mol. Endocrinol. 1998, 12: 1830-1845).

It has been demonstrated that GPR49 is over-expressed (3-fold) in almost half of hepatocellular carcinomas compared to surrounding tissues (Yamamoto et al. Hepatology, 2003, 37: 528-533). This high-level expression correlated with the presence of β -catenin mutations in hepatocellular cell-lines. Additionally, a strong GPR49 expression in colon cancer cells was demonstrated, which is dependent upon TCF/ β -catenin signalling.

Moreover, the expression in colon carcinoma cells

was reduced 3-fold following inhibition of TCF/ β -catenin signalling (Table 1/Figure 2B).

The GPR49 mRNA and protein sequences are given in Figure 20.

5

EXAMPLE 5

EPHB4

EPHB4 belongs to the EPH-related receptor
10 tyrosine kinase family, which has important roles in many cellular processes, including neural development, angiogenesis, vascular network assembly and proliferation. Genetic studies using targeted mutagenesis in mice reveals that EPHB4, together with the ligands ephrin-B1 and ephrin-
15 B2 is essential for the normal development of embryonic vascular networks into arteries, veins and capillaries. EPHB4 knockout mice die during embryogenesis, probably as a result of failed cardiovascular development. EPHB2 and EPHB3 are also expressed on veins and/or arteries and
20 combined loss of expression also leads to vascular defects, although less pronounced than those seen for EPHB4. There is evidence to suggest that EPH family members are upregulated as blood vessels invade tumours, linking EPH function with angiogenesis. EPHB4 is reported to be over-
25 expressed in ovarian cancer, endometrial tumors, choriocarcinoma, teratocarcinoma and colon cancer. Preliminary evidence indicates that this elevated expression in colon cancer is a direct result of high level TCF/ β -catenin signalling, suggesting that EPHB4, as well as
30 EPHB2 and EPHB3 are TCF target genes. Given the likely roles of EPHB4 (and other members) in tumour development, it makes an attractive target for antibody-based therapies.

The EPHB4 cDNA and protein sequence are given in

Figure 21.

EPHB4 is a receptor tyrosine kinase, class VIII with a vestigial Ig-I domain, a single cysteine rich region and two FNIII domains in the extracellular region. EPHB4 is abundantly expressed in placenta and veins (but not arteries) and in a wide range of primary tissues.

Northern analysis revealed EPHB4 to be a target of TCF/ β -catenin in colon carcinoma cells (Fig. 2B). In accordance with this result, it was also shown that EPHB4 was highly expressed in intestinal crypts and over-expressed in colorectal polyps (Figure 7).

EXAMPLE 6

GPX-2

GPX-2 is a member of the family of selenium-dependent glutathione peroxidases, which are generally thought to have an anti-oxidant function, protecting tissues from reactive oxygen species. GPX-2 is the least reliant of the family members on Selenium for expression, due mainly to the stability of its mRNA during Selenium depletion. It is highly expressed in the intestine and liver and some reports indicate also a lower level expression in the epithelium of the oesophagus. In the mouse, GPX-2 maps close to a colon cancer susceptibility locus and high expression levels correlate with resistance to colon cancer. GPX-2 protein levels have also been shown to increase in intestinal adenomas compared to adjacent normal mucosa. This has led to speculation that cells may upregulate GPX-2 (via transcriptional response to reactive oxygen species) in the presence of reactive oxygen species to protect against cancer by preventing the DNA damage that would otherwise occur. According to the present invention

it was observed that GPX-2 expression is dependent upon TCF/ β -catenin signalling in colon cancer and recent studies showing high-level expression in intestinal crypts and early stage colon cancer indicate that it may in fact play a role in regulating cell growth and differentiation, or may serve to protect the developing cancer tissue from oxidative stress.

The GPX2 mRNA sequence and protein sequence are given in Figure 22.

GPX-2 is highly expressed in the intestine, colon, stomach, liver and galbladder. Expression reduced 3-fold in microarray experiments following inhibition of TCF/ β -catenin signalling (table 1). This was confirmed by Northern blot analysis (Figure 2B).

Semi-quantitative and Q-PCR analysis of GPX-2 expression levels in a panel of human cancer cell-lines demonstrated a significant expression in cancer cell-lines of various origin, with particularly high levels evident in colon carcinoma cell-lines LS174T, HT29, SW480, the lung cancer cell-line A549 and the breast cancer cell-line MDAMB351 (Figure 8 and Figure 9A).

EXAMPLE 7

RGMR

The Human Repulsive Guidance Molecule-Related (RGMR) Gene (RGMR) is predicted to encode a 47KDa GPI-anchored glycoprotein which is closely related to a family of molecules designated repulsive guidance molecules (RGM) present in humans, mice, chicken and Xenopus (Figure 29). The RGM designation was assigned following the discovery of the role chicken RGM has in axon guidance during nervous system development. A murine orthologue of human RGMR

exists, indicating the existence of an RGM sub-family (which was termed RGMR). No RGMR orthologue has been identified in the chicken, suggesting that the RGMR genes may have arisen via duplication of the RGM gene during evolution.

Expression of human RGMR was found to be dependent on active TCF/ β -catenin signaling in colon cancer cells (Table 1, Figure 2B).

It was previously shown that another family of repulsive guidance proteins, the EPHB tyrosine kinase receptors, are targets of TCF/ β -catenin signaling and these proteins are essential for the correct positioning of cell-populations during development of the small intestine. RGMR proteins may have similar roles to play in this developmental process.

The hRGMR cDNA sequence and hRGMR protein sequence are given in Figure 23. RGMR corresponds to image sequence 376697 in Table 1.

This image sequence is 2kb downstream of the actual predicted coding sequence of RGMR.

Semi-quantitative and Q-PCR analysis of RGMR expression levels in a panel of human cancer cell-lines demonstrated high-level expression in selected colon, lung and prostate cell-lines, with highest levels evident in the colon cancer cell-line LS174T (Figure 8 and 9C).

EXAMPLE 8

Tspan5

Tspan 5/NET4 is a member of the tetraspan superfamily of proteins, which are characterized by four transmembrane domains and two extracellular regions. Within this superfamily is a sub-family of highly related proteins

referred to as NET proteins (for New Est Tetraspan), of which there are currently more than 20 members. The function of these NET proteins is largely unknown, although it has been suggested that they may group together specific cell-surface proteins including kinases to promote the formation of stable functional signalling complexes. General functions are considered to include regulation of cell development, activation, growth and motility. The limited data currently available suggests distinct roles for individual tetraspan molecules, with some having inhibitory roles in cancer and others apparently being expressed at high levels in cancer cells. Tetraspan 5 is one such family member that is expressed at high levels in colon cancer, probably as a direct consequence of constitutive TCF/ β -catenin signalling. Expression levels drop 3-fold following inhibition of TCF/ β -catenin signalling in colon cancer cell-lines (Table 1).

The cDNA and protein sequence of Tspan5 are given in Figure 24.

Figure 16 shows a schematic model of the domain structure of Tspan5, demonstrating 4 transmembrane domains and two large extracellular loops.

The mouse homologue is mainly expressed in brain tissue, but also in heart, kidney, testis and weakly in liver. EST data on human Tspan5 indicate a similar expression pattern.

Semi-quantitative and Q-PCR analysis of Tspan5 expression levels in a panel of human cancer cell-lines demonstrated high level expression in selected colon, lung and prostate cancer cell-lines, with highest levels evident in cancer cell-line HCT116 (Figure 8 and 9D).

EXAMPLE 9

Production of polyclonal antibodies

Polyclonal antibodies directed against selected
 5 target surface-expressed target antigens (EPHB2, EPHB3,
 Tspan5 and RGMR) were generated by immunization of rabbits
 with gene-specific peptides predicted to be immunogenic and
 adopt a conformation similar to that of the corresponding
 region of the native protein (Software such as 'antigen
 10 prediction' within the EMBOSS package of the UK HGMP
 Resource Centre web-site was employed here). The presence
 of antibodies directed against the target antigens was
 confirmed by screening sera from immunized rabbits against
 Cos7 cell transfectants expressing the appropriate full-
 15 length target protein.

The following peptides were synthesized and
 conjugated to BSA (bovine serum albumin) for rabbit
 immunization:

20	EPHB2	Peptide 1:	H-YEKELSEYNATALKSPC-NH2
		Peptide 2:	H-PFSPQFASVNC-NH2
	EPHB3	Peptide 1:	H-PGSYKAKQGEGPC-NH2
		Peptide 2:	H-CQMNSVQLDGLPDARY-OH
25			
	TSPAN5	Peptide 1:	H-CGYDARQKPEVDQQ-OH
		Peptide 2:	H-CKGVLSNISSITDLGGFD-OH
	RGMR	Peptide 1:	H-HSALEDVEALHPRKERC-NH2
30		Peptide 2:	H-CNYHSHAGAREHRRGD-OH

The antibodies can be used as colon cancer
 therapeutics via modulation of target protein function at

the cell-surface via inhibition of ligand binding or inappropriate activation of downstream signaling cascades in the cancer cells. Given the high-levels of expression of these target proteins in colon cancer, these antibodies
5 will also be useful as diagnostic tools for colon cancer incidence and progression.

CLAIMS

1. Use of inhibitors of the expressed proteins,
5 or peptides derived therefrom, of TCF target genes whose expression is regulated by TCF/ β -catenin complexes for the preparation of a therapeutical composition for the treatment of cancers in which TCF/ β -catenin signalling is deregulated.

10 2. Use as claimed in claim 1, wherein the inhibitors are antibodies or derivatives thereof directed against the expression products of the target genes that are expressed on the cell membrane.

3. Use as claimed in claim 1 or 2, wherein the
15 antibodies or derivatives thereof are directed against a peptide, which is chosen from the group consisting of:
H-YEKELSEYNATALKSPC-NH₂; H-PFSPQFASVNC-NH₂;
H-PGSYKAKQGEGPC-NH₂; H-CQMNSVQLDGLPDARY-OH;
H-CGYDARQKPEVDQQ-OH; H-CKGVLSNISSITDLGGFD-OH; H-
20 HSALEDVEALHPRKERC-NH₂; and H-CNYHSHAGAREHRRGD-OH.

4. Use as claimed in claim 1, 2 or 3, wherein the derivatives are selected from the group consisting of scFv fragments, Fab fragments, chimeric antibodies, bifunctional antibodies, and other antibody-derived molecules.

25 5. Use as claimed in claim 1, wherein the inhibitors are small molecules interfering with the biological activity of the protein expressed by the target gene.

6. Use of inhibitors of the mRNA transcripts of
30 TCF target genes whose expression is regulated by TCF/ β -catenin complexes for the preparation of a therapeutical composition for the treatment of cancers in which TCF/ β -catenin signalling is deregulated.

7. Use as claimed in claim 6, wherein the inhibitors are antisense molecules, in particular antisense RNA or antisense oligodeoxynucleotides.

8. Use as claimed in claim 6, wherein the
5 inhibitors are double stranded RNA molecules for RNA interference.

9. Use as claimed in claim 1, wherein the treatment comprises gene therapy.

10. Use as claimed in any one of the claims 1-9,
10 wherein the therapeutical composition is for treatment of Familial Adenomatous Polyposis (FAP).

11. Use as claimed in any one of the claims 1-9, wherein the therapeutical composition is for treatment of colorectal cancer.

12. Use as claimed in any one of the claims 1-9,
15 wherein the therapeutical composition is for treatment of melanomas.

13. Use of TCF target genes whose expression is regulated by TCF/ β -catenin complexes for the diagnosis of
20 cancers in which TCF/ β -catenin signalling is deregulated.

14. Use as claimed in claim 13, wherein the diagnosis is performed by means of histological analysis of tissue specimens using specific antibodies directed against target gene products, and/or in situ hybridization analysis
25 of TCF/ β -catenin target gene expression levels in tissue specimens using specific RNA probes directed against TCF/ β -catenin target gene sequences.

15. Use as claimed in any one of the claims 1-14, wherein the target gene is selected from the group
30 consisting of CD44, KIT, G protein-coupled receptor 49 (GPR49), Solute Carrier Family 12 member 2 (SLC12A2), Solute Carrier Family 7 member 5, Claudin 1 (CLDN1), SSTR serine threonine kinase, FYN oncogene, EPHB2 receptor

tyrosine kinase, EPHB3 receptor tyrosine kinase, EPHB4 receptor tyrosine kinase, ETS2, c-Myc, MYB, ID3, POLE3, Bone Morphogenetic Protein 4 (BMP4), Kit ligand (KITLG), GPX2, GNG2, CDCA7, ENC1, the gene identified with Celera ID
5 hCG40185, the gene identified with Celera ID hCG1645335, the gene represented by IMAGE clone 1871074, the gene identified with Celera ID hCG27486, the gene represented by IMAGE clone 294873, the gene represented by IMAGE clone 940994, the gene identified with Celera ID 39573, the gene
10 represented by IMAGE clone 753028, the gene identified with Celera ID hCG37727, the gene identified with Celera ID hCG40978, and the gene identified with Celera ID hCG1811066.

16. Use as claimed in any one of the claims 1-15,
15 wherein the target gene is CD44, comprising a cDNA sequence, which is at least 90% homologous to the cDNA sequence shown in Figure 17 (SEQ. ID. No 1), Figure 18 or Figure 19.

17. Use as claimed in any one of the claims 1-15,
20 wherein the target gene is GPR49, comprising a cDNA sequence which is at least 90% homologous to the sequence shown in Figure 20 (SEQ. ID. No 3).

18. Use as claimed in any one of the claims 1-15, wherein the target gene is EPHB4, comprising a cDNA
25 sequence which is at least 90% homologous to the sequence shown in Figure 21 (SEQ. ID. No 5).

19. Use as claimed in any one of the claims 1-15, wherein the target gene is GPX2, comprising a cDNA sequence which is at least 90% homologous to the sequence shown in
30 Figure 22 (SEQ. ID. No 7).

20. Use as claimed in any one of the claims 1-15, wherein the target gene is RGMR, comprising a cDNA sequence which is at least 90% homologous to the sequence shown in

Figure 23 (SEQ. ID. No 9).

21. Use as claimed in any one of the claims 1-15,
wherein the target gene is Tspan5, represented by a
sequence which is at least 90% homologous to the sequence
5 shown in Figure 24 (SEQ. ID. No 11).

22. Use as claimed in any of the claims 1-15
wherein the expressed protein comprises a sequence which is
at least 90% homologous to the protein sequences as shown
in Figure 17 or 18.

10 23. Use as claimed in any of the claims 1-15
wherein the expressed protein comprises a sequence which is
at least 90% homologous to the protein sequences of Figure
20 (SEQ ID No. 4).

24. Use as claimed in any of the claims 1-15
15 wherein the expressed protein comprises a sequence which is
at least 90% homologous to the protein sequences of Figure
21 (SEQ ID No. 6).

25. Use as claimed in any of the claims 1-15
wherein the expressed protein comprises a sequence which is
20 at least 90% homologous to the protein sequences of Figure
22 (SEQ ID No. 8).

26. Use as claimed in any of the claims 1-15
wherein the expressed protein comprises a sequence which is
at least 90% homologous to the protein sequences of Figure
25 23 (SEQ ID No. 10).

27. Use as claimed in any of the claims 1-15
wherein the expressed protein comprises a sequence which is
at least 90% homologous to the protein sequences of Figure
24 (SEQ ID No. 12).

30 28. Inhibitor compound directed against the
expressed proteins, or peptides derived therefrom, of a TCF
target gene the expression of which is regulated by TCF/ β -
catenin complexes for use in the treatment of colorectal

cancer.

29. Inhibitor compound as claimed in claim 28,
which is an antibody or derivatives thereof directed
against the expression products of a target gene that is
5 expressed on the cell membrane.

30. Inhibitor compound as claimed in claim 29
wherein the antibodies or derivatives thereof are directed
against a peptide, which is chosen from the group
consisting of:

10 H-YEKELSEYNATALKSPC-NH₂; H-PFSPQFASVNC-NH₂;
H-PGSYKAKQGEGPC-NH₂; H-CQMNSVQLDGLPDARY-OH;
H-CGYDARQKPEVDQQ-OH; H-CKGVLSNISSITDLGGFD-OH; H-
HSALEDVEALHPRKERC-NH₂; and H-CNYHSHAGAREHRRGD-OH.

31. Inhibitor compound as claimed in claim 29 or
15 30, wherein the derivative is selected from the group
consisting of scFv fragments, Fab fragments, chimeric
antibodies, bifunctional antibodies, or other antibody-
derived molecules.

32. Inhibitor compound as claimed in claim 28,
20 which is a small molecule interfering with the biological
activity of the protein expressed by the target gene.

33. Inhibitor compound directed against the
transcription product (mRNA) of a TCF target gene the
expression of which is regulated by TCF/ β -catenin complexes
25 for use in the treatment of colorectal cancer.

34. Inhibitor compound as claimed in claim 33,
which is an antisense molecule, in particular an antisense
RNA or an antisense oligodeoxynucleotide.

35. Inhibitor compound as claimed in claim 34,
30 which is a double stranded RNA molecule for RNA
interference.

36. Inhibitor compound as claimed in any one of
the claims 28-35, wherein the target gene is selected from

the group consisting of CD44, KIT, G protein-coupled receptor 49 (GPR49), Solute Carrier Family 12 member 2 (SLC12A2), Solute Carrier Family 7 member 5, Claudin 1 (CLDN1), SSTK serine threonine kinase, FYN oncogene, EPHB2
5 receptor tyrosine kinase, EPHB3 receptor tyrosine kinase, EPHB4 receptor tyrosine kinase, ETS2, c-Myc, MYB, ID3, POLE3, Bone Morphogenetic Protein 4 (BMP4), Kit ligand (KITLG), GPX2, GNG2, CDCA7, ENC1, the gene identified with Celera ID hCG40185, the gene identified with Celera ID
10 hCG1645335, the gene represented by IMAGE clone 1871074, the gene identified with Celera ID hCG27486, the gene represented by IMAGE clone 294873, the gene represented by IMAGE clone 940994, the gene identified with Celera ID 39573, the gene represented by IMAGE clone 753028, the gene
15 identified with Celera ID hCG37727, the gene identified with Celera ID hCG40978, and the gene identified with Celera ID hCG1811066.

37. Inhibitor compound as claimed in any one of the claims 28-35, wherein the target gene is CD44,
20 comprising a cDNA sequence which is at least 90% homologous to the sequence shown in Figure 17 (SEQ. ID. No 1), Figure 18, or Figure 19.

38. Inhibitor compound as claimed in any one of the claims 28-35, wherein the target gene is GPR49,
25 comprising a cDNA sequence which is at least 90% homologous to the sequence shown in Figure 20 (SEQ. ID. No 3).

39. Inhibitor compound as claimed in any one of the claims 28-35, wherein the target gene is EPHB4,
comprising a cDNA sequence which is at least 90% homologous
30 to the sequence shown in Figure 21 (SEQ. ID. No 5).

40. Inhibitor compound as claimed in any one of the claims 28-35, wherein the target gene is GPX2,
comprising a cDNA sequence which is at least 90% homologous

to the sequence shown in Figure 22 (SEQ. ID. No 7).

41. Inhibitor compound as claimed in any one of the claims 28-35, wherein the target gene is RGMR, comprising a cDNA sequence which is at least 90% homologous
5 to the sequence shown in Figure 23 (SEQ. ID. No 9).

42. Inhibitor compound Inhibitor compound as claimed in any one of the claims 28-35, wherein the target gene is Tspan5, represented by a sequence which is at least 90% homologous to the sequence shown in Figure 24 (SEQ. ID.
10 No 11).

43. Inhibitor compound as claimed in any of the claims 28-35 wherein the expressed protein comprises a sequence which is at least 90% homologous to the protein sequences of Figure 17 or 18.

15 44. Inhibitor compound as claimed in any of the claims 28-35 wherein the expressed protein comprises a sequence which is at least 90% homologous to the protein sequences of Figure 20 (SEQ ID No. 4).

20 45. Inhibitor compound as claimed in any of the claims 28-35 wherein the expressed protein comprises a sequence which is at least 90% homologous to the protein sequences of Figure 21 (SEQ ID No. 6).

25 46. Inhibitor compound as claimed in any of the claims 28-35 wherein the expressed protein comprises a sequence which is at least 90% homologous to the protein sequences of Figure 22 (SEQ ID No. 8).

30 47. Inhibitor compound as claimed in any of the claims 28-35 wherein the expressed protein comprises a sequence which is at least 90% homologous to the protein sequences of Figure 23 (SEQ ID No. 10).

48. Inhibitor compound as claimed in any of the claims 1-15 wherein the expressed protein comprises a sequence which is at least 90% homologous to the protein

sequences of Figure 24 (SEQ ID No. 12).

49. Diagnostic agent for diagnosing cancers in which TCF/ β -catenin signaling is deregulated.

50. Diagnostic agent as claimed in claim 49,
5 which is a specific antibody directed against the expressed protein of a TCF/ β -catenin target gene or an RNA probe specific for a TCF/ β -catenin target gene sequence.

51. Therapeutical composition for the treatment of cancers in which the TCF/ β -catenin signaling is
10 deregulated, comprising a suitable excipient, carrier and/or diluent and one or more inhibitor compounds as claimed in claims 28-48.

52. Diagnostic composition for the diagnosis of cancers in which the TCF/ β -catenin signaling is
15 deregulated, comprising a suitable excipient, carrier and/or diluent and one or more diagnostic compounds as claimed in claim 49 or 50.

53. Compositions as claimed in claim 51 or 52, wherein the cancer is colorectal cancer, melanoma or
20 Familial Adenomatous Polyposis (FAP).

54. Method for the development of therapeutic inhibitor compounds as claimed in claims 28-48, which method comprises the steps:

a) identification of genes regulated by TCF/ β -
25 catenin in colon carcinoma cells, in particular by using microarray technologies;

b) validation of one or more of the identified genes as potential target gene(s) for the therapeutic compound by one or more of the following methods:

30 - confirmation of the identified gene by Northern Blot analysis in colon carcinoma cell-lines;
- determination of the expression profile of

the identified gene in human colorectal tumors and normal tissue;

- determination of the functional importance of the identified target genes for colorectal cancer;

c) production of the expression product of the target gene; and

d) use of the expression product of the target gene for the production or design of a therapeutic compound.

55. Method as claimed in claim 54, wherein the target gene identified in step a) is selected from the group consisting of CD44, KIT, G protein-coupled receptor 49 (GPR49), Solute Carrier Family 12 member 2 (SLC12A2), Solute Carrier Family 7 member 5, Claudin 1 (CLDN1), SSTK serine threonine kinase, FYN oncogene, EPHB2 receptor tyrosine kinase, EPHB3 receptor tyrosine kinase, EPHB4 receptor tyrosine kinase, ETS2, c-Myc, MYB, ID3, POLE3, Bone Morphogenetic Protein 4 (BMP4), Kit ligand (KITLG), GPX2, GNG2, CDCA7, ENC1, the gene identified with Celera ID hCG40185, the gene identified with Celera ID hCG1645335, the gene represented by IMAGE clone 1871074, the gene identified with Celera ID hCG27486, the gene represented by IMAGE clone 294873, the gene represented by IMAGE clone 940994, the gene identified with Celera ID 39573, the gene represented by IMAGE clone 753028, the gene identified with Celera ID hCG37727, the gene identified with Celera ID hCG40978, and the gene identified with Celera ID hCG1811066.

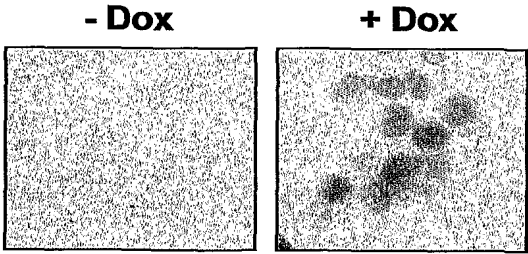


FIG. 1A

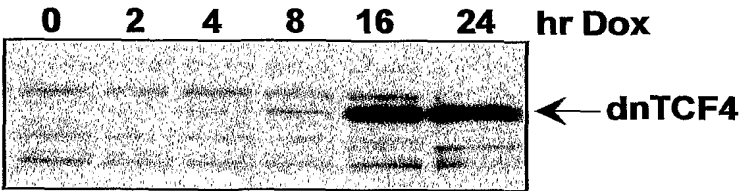


FIG. 1B

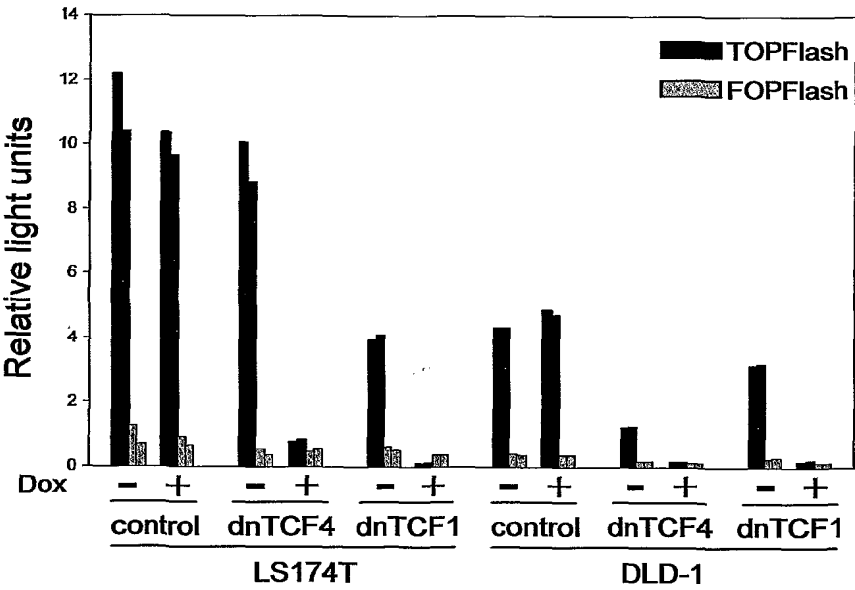


FIG. 1C

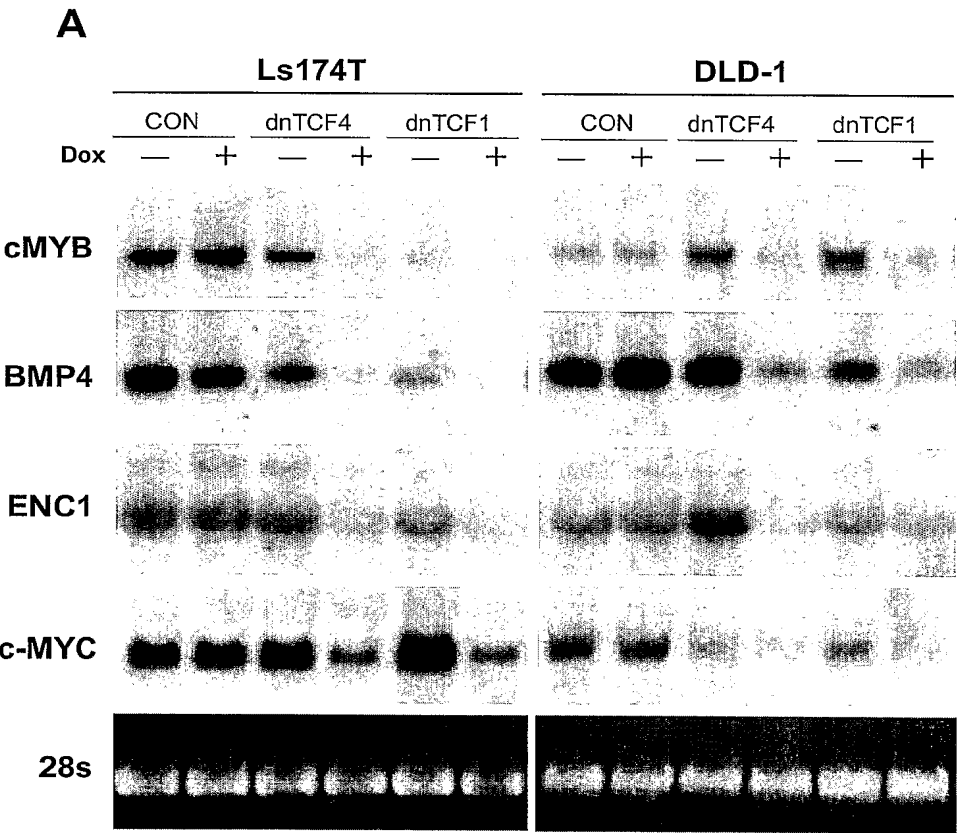
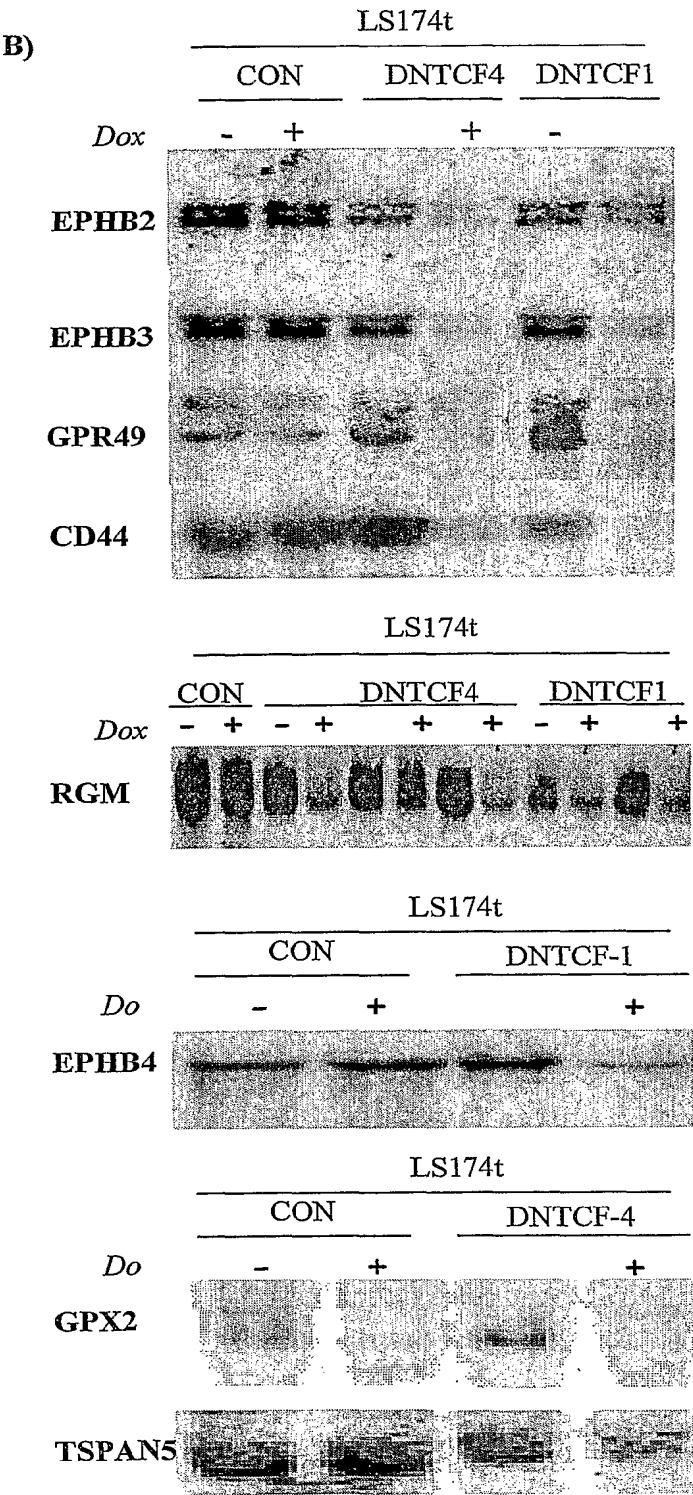


FIG. 2A



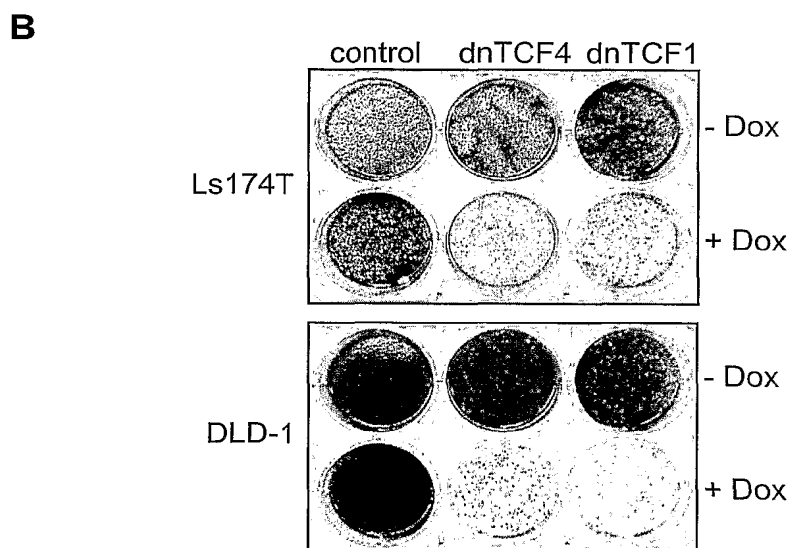
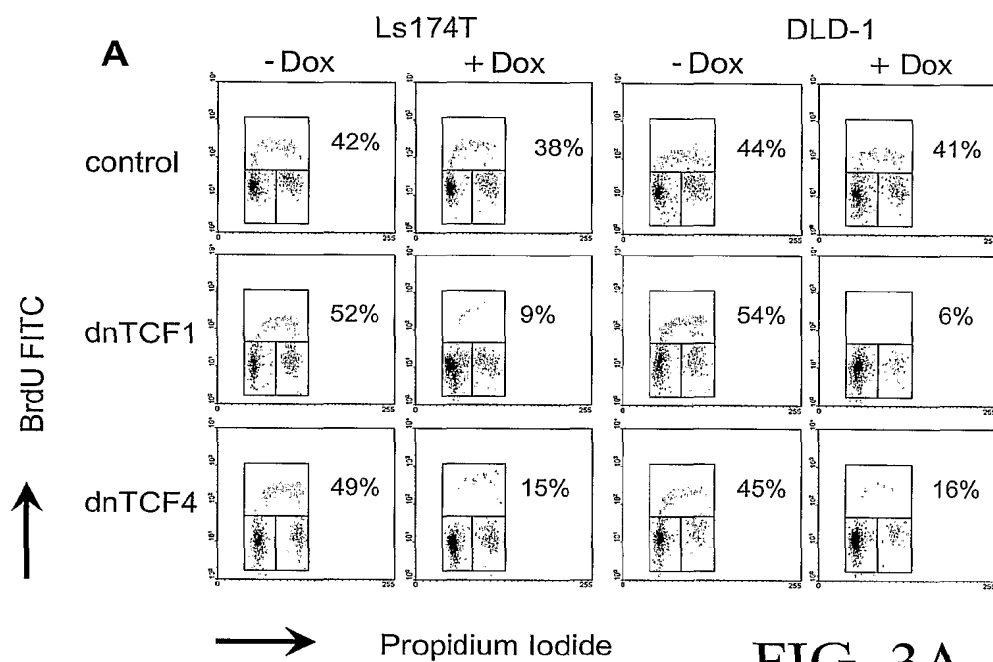


FIG. 3B

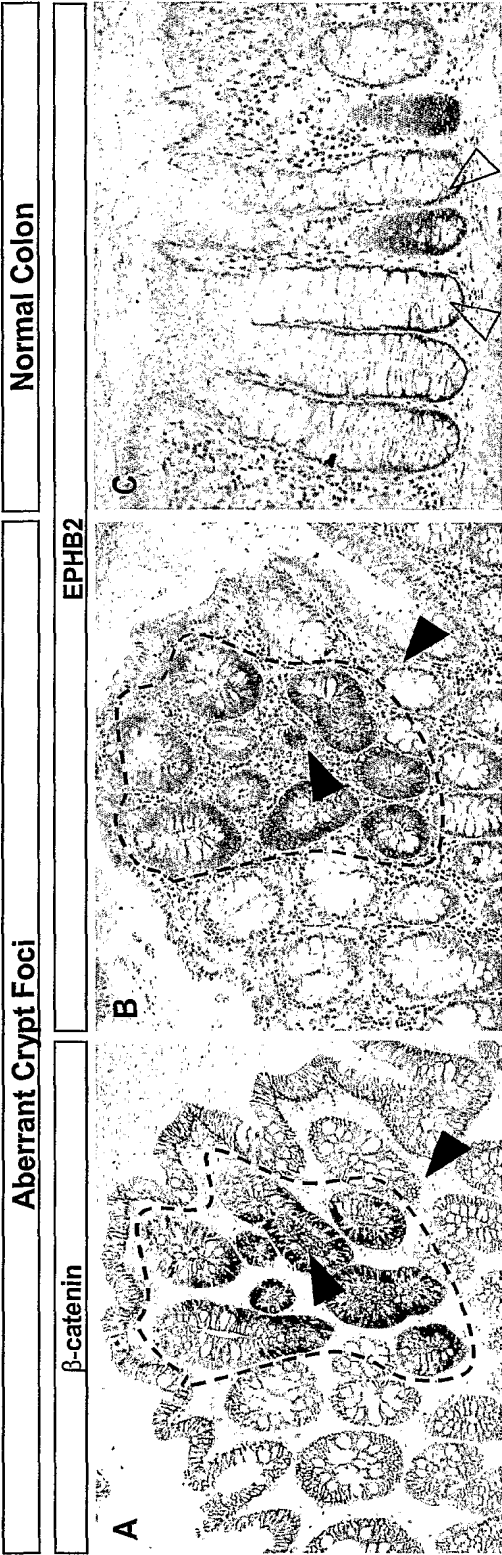


FIG. 4A

FIG. 4B

FIG. 4C



FIG. 5B

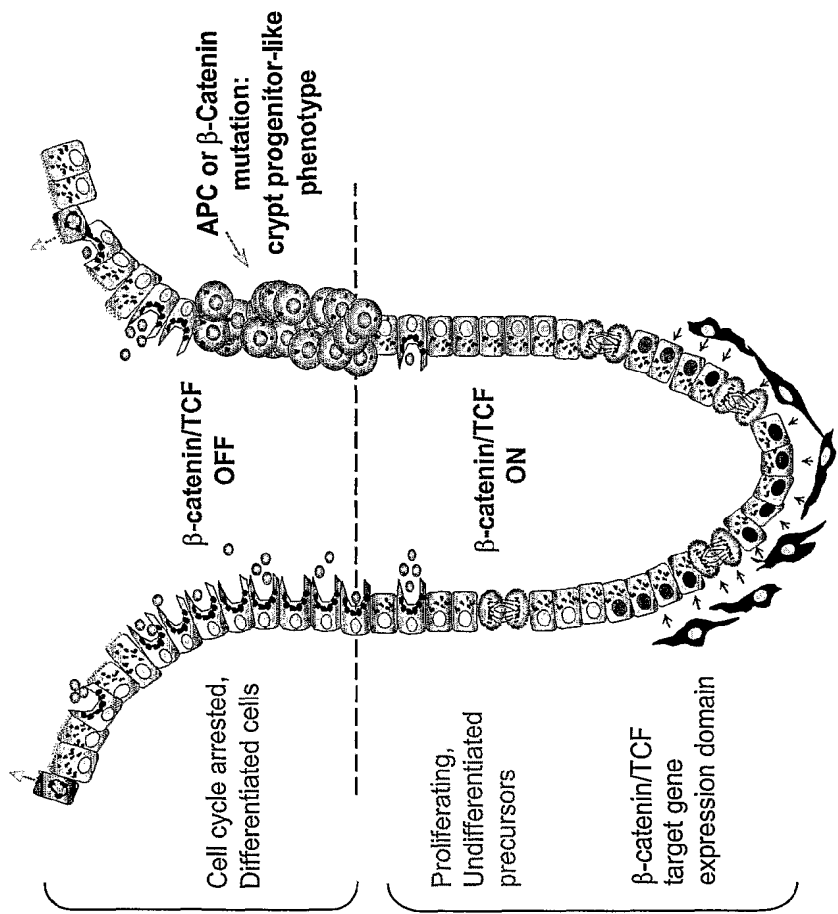


FIG. 5A

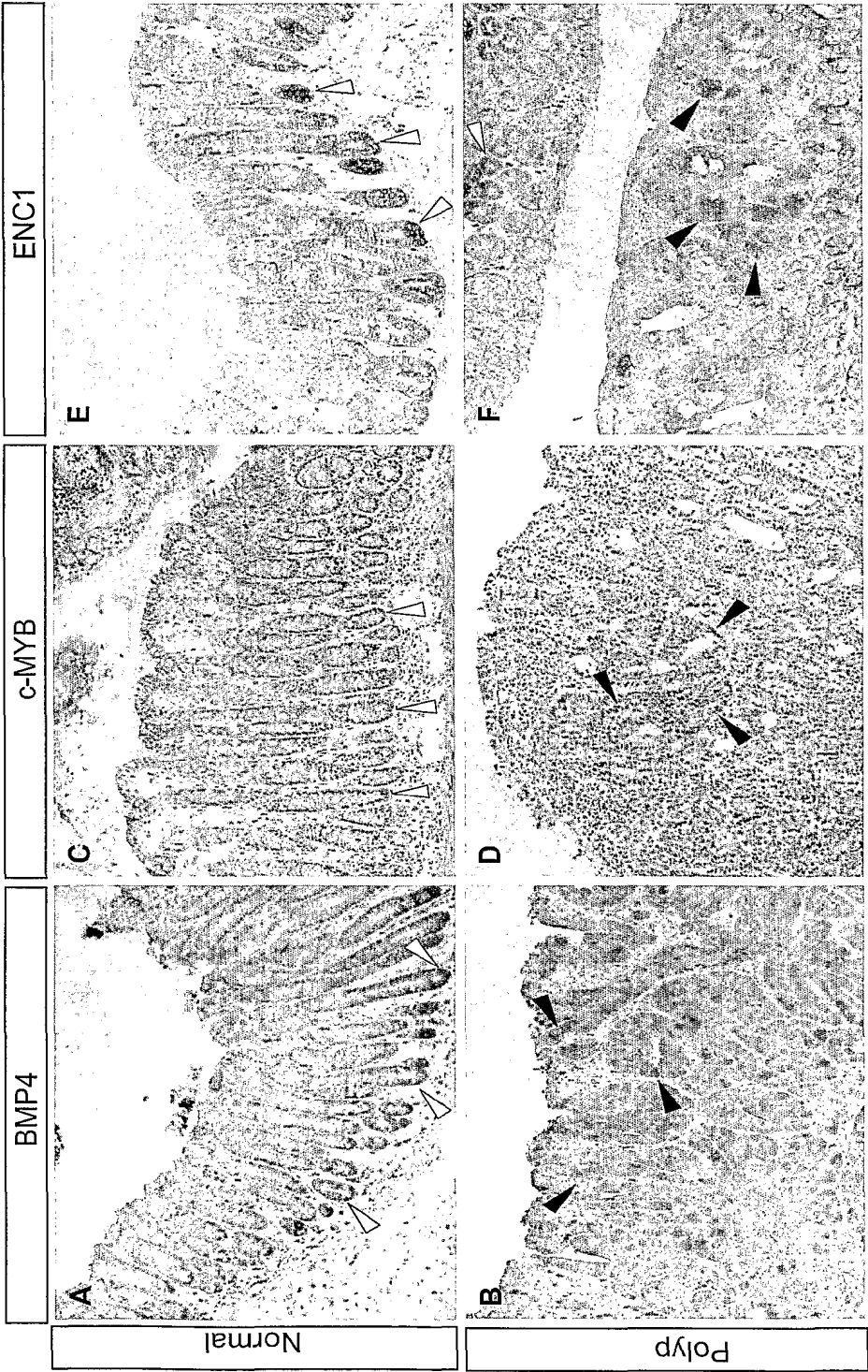
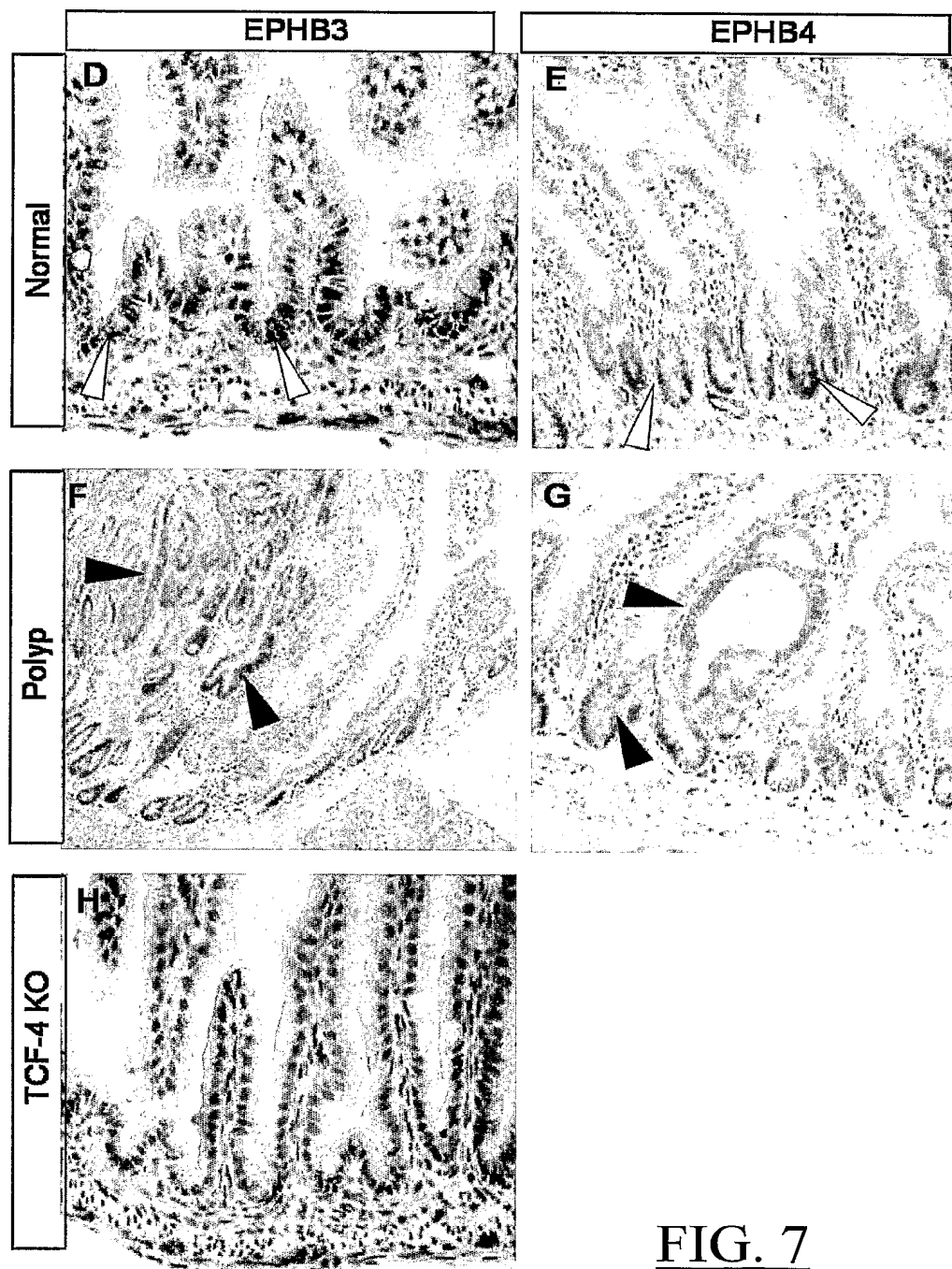


FIG. 6

**FIG. 7**

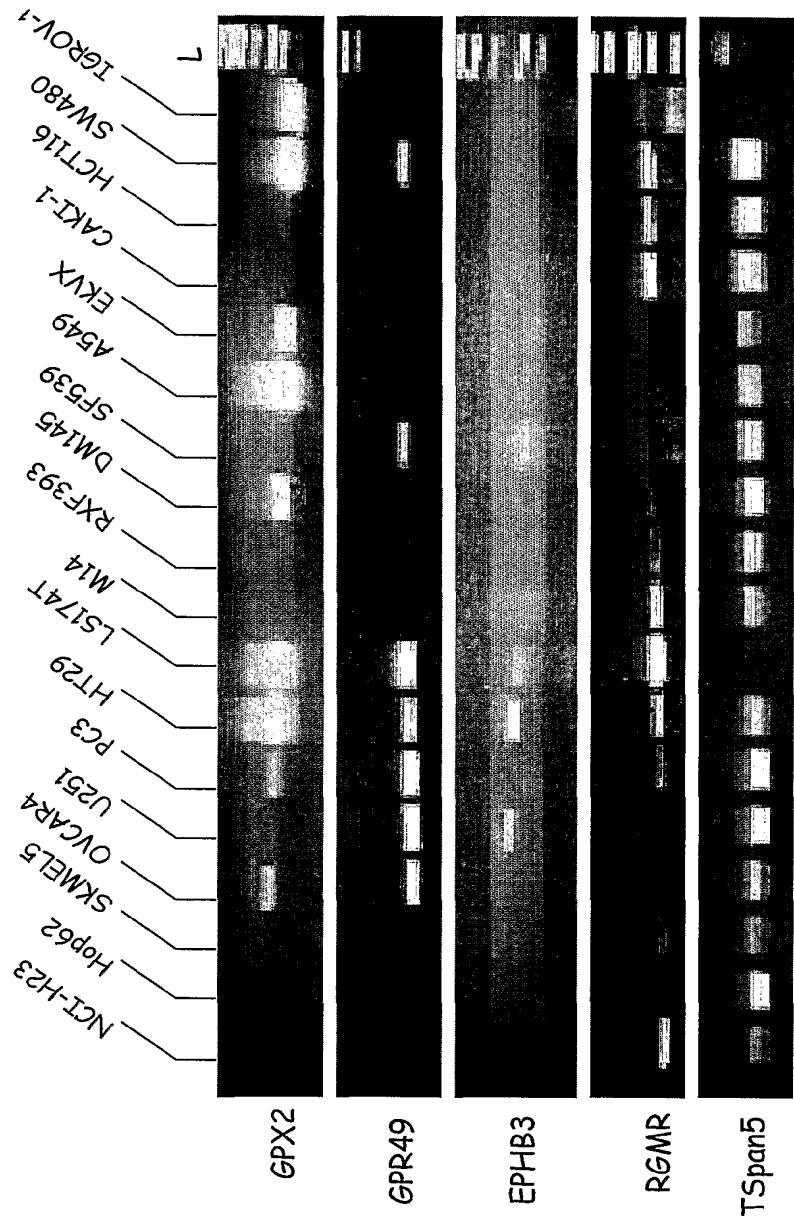
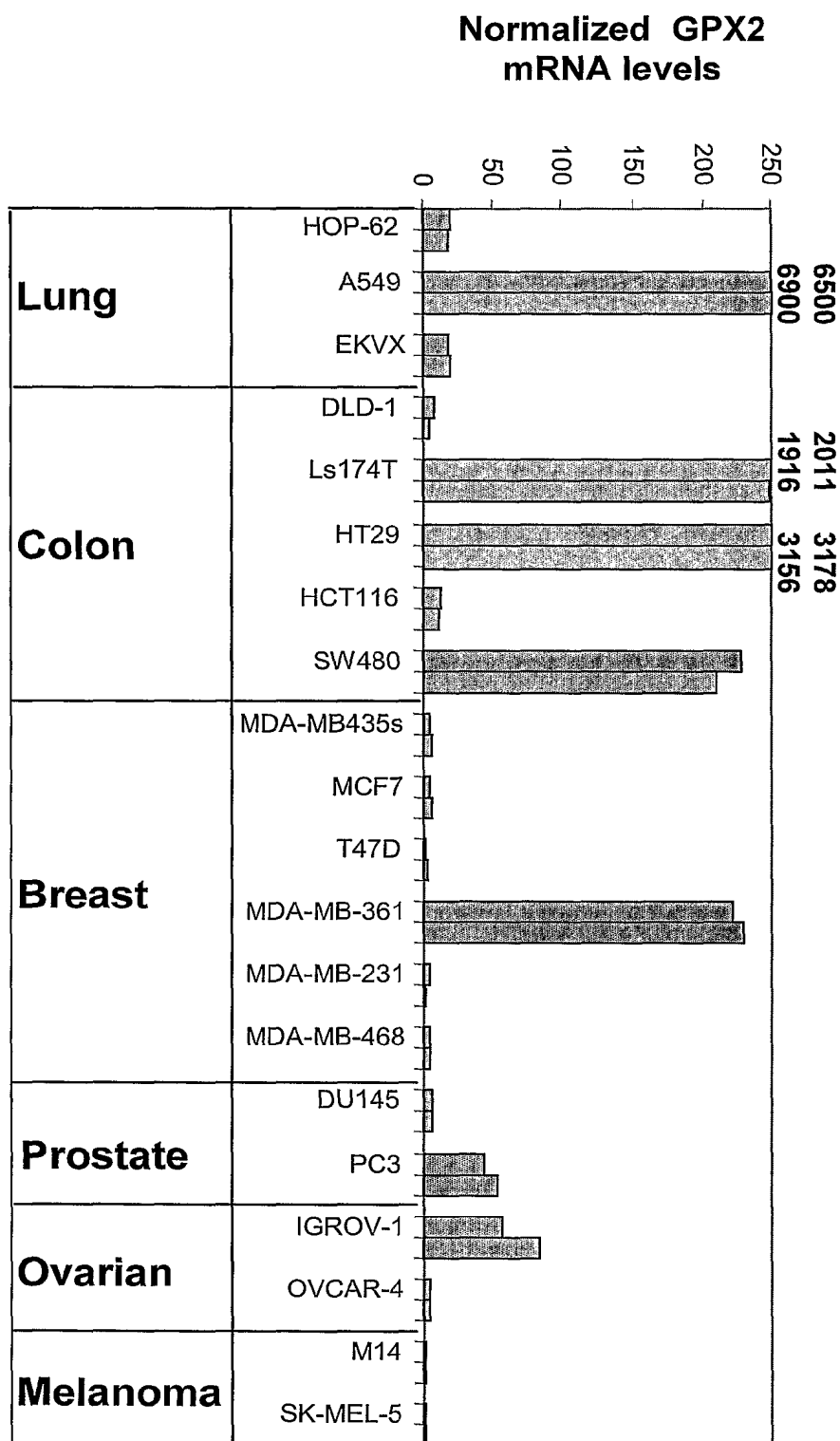


FIG. 8A

Cancer Type	Cell-Line	GPX2 Expression Level	GPR49 Expression Level	EPHB3 Expression Level	RGMR Expression Level	TSpan 5 Expression Level
Lung	NCL-H23	+	-	+	++	+
	Hop62	+	-	+	+	++
	A549	++++	-	+	+	+
	EKVX	+++	-	+	+	+
Ovarian	OVCAR-4	++	++	-	+	+
	IGROV-1	+++	-	-	+	-
Central Nervous System	U251	+	+++	++	+	+++
	SF539	-	+	++	-	++
Skin	SKMEL5	-	-	-	+	+
	M14	-	-	+	++	+
Colon	HT29	++++	++	+++	++	+
	LS174T	++++	+++	++	+++	ND
	SW480	+++	++	-	+++	+++
	HCT116	+	-	-	++	++
Prostate	PC3	++	+++	-	+	+++
	DM145	+++	-	-	+	+++
Kidney	RXF393	-	-	-	+	+
	CAKI-1	-	-	-	+++	++

FIG. 8B

**FIG. 9A**

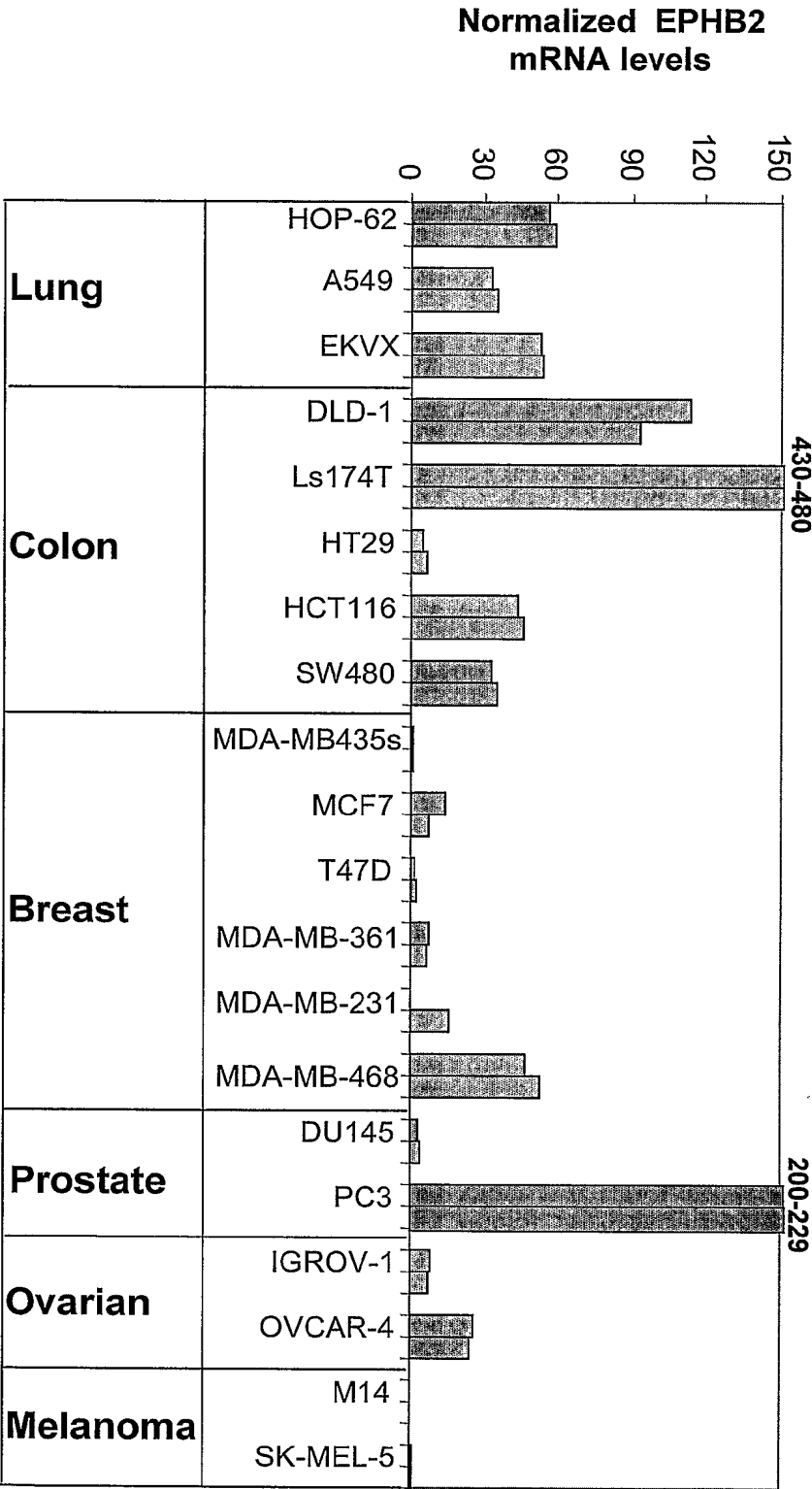
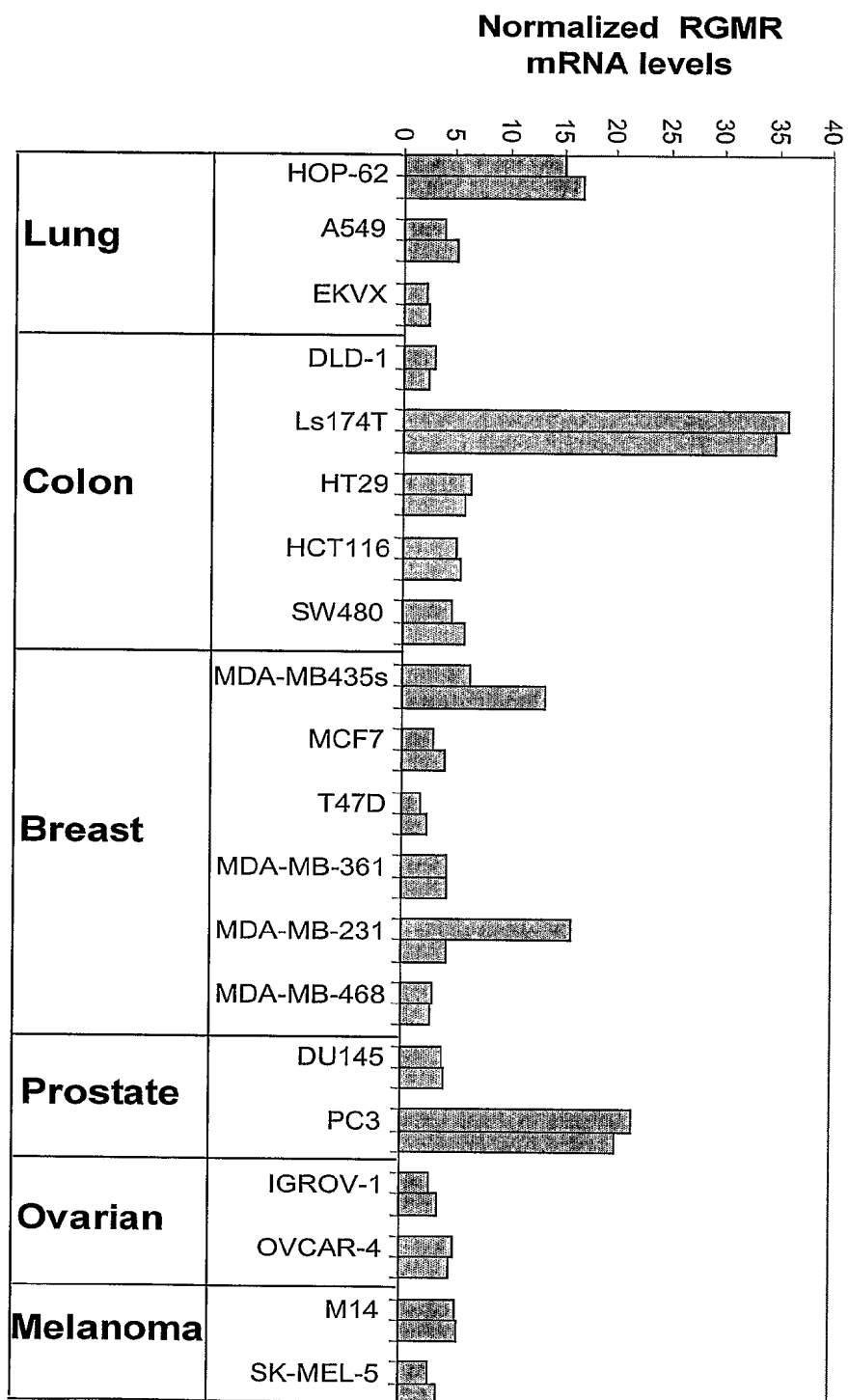
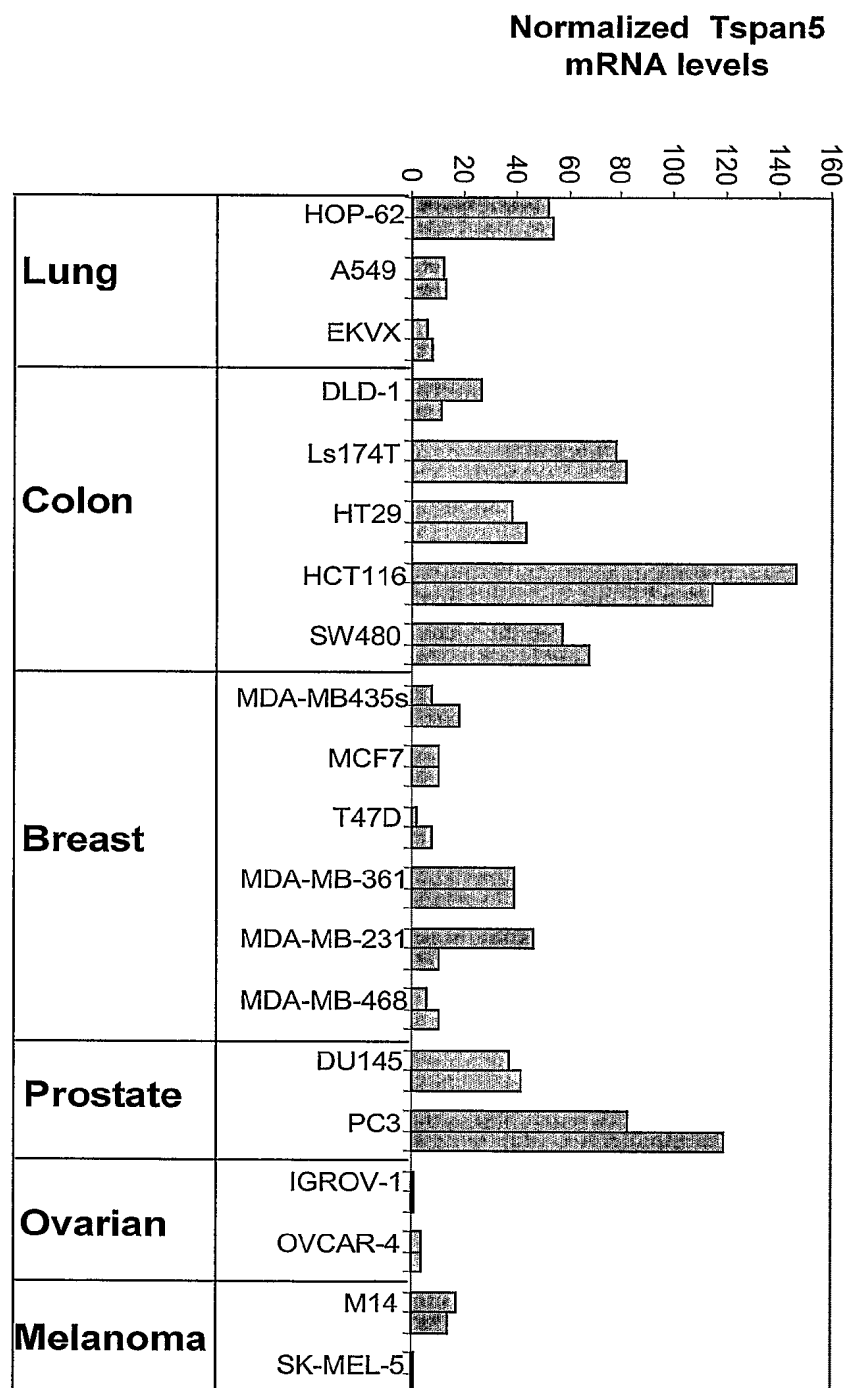


FIG. 9B

**FIG. 9C**

**FIG. 9D**

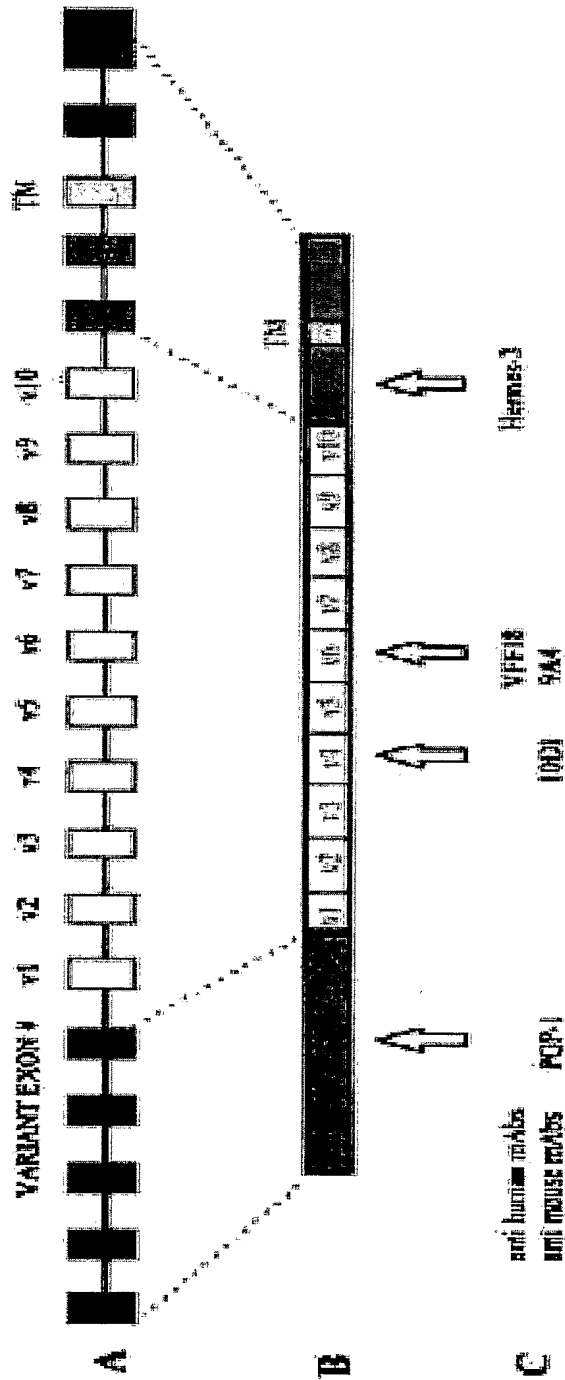


FIG. 10

[illegible]

FIG. 11

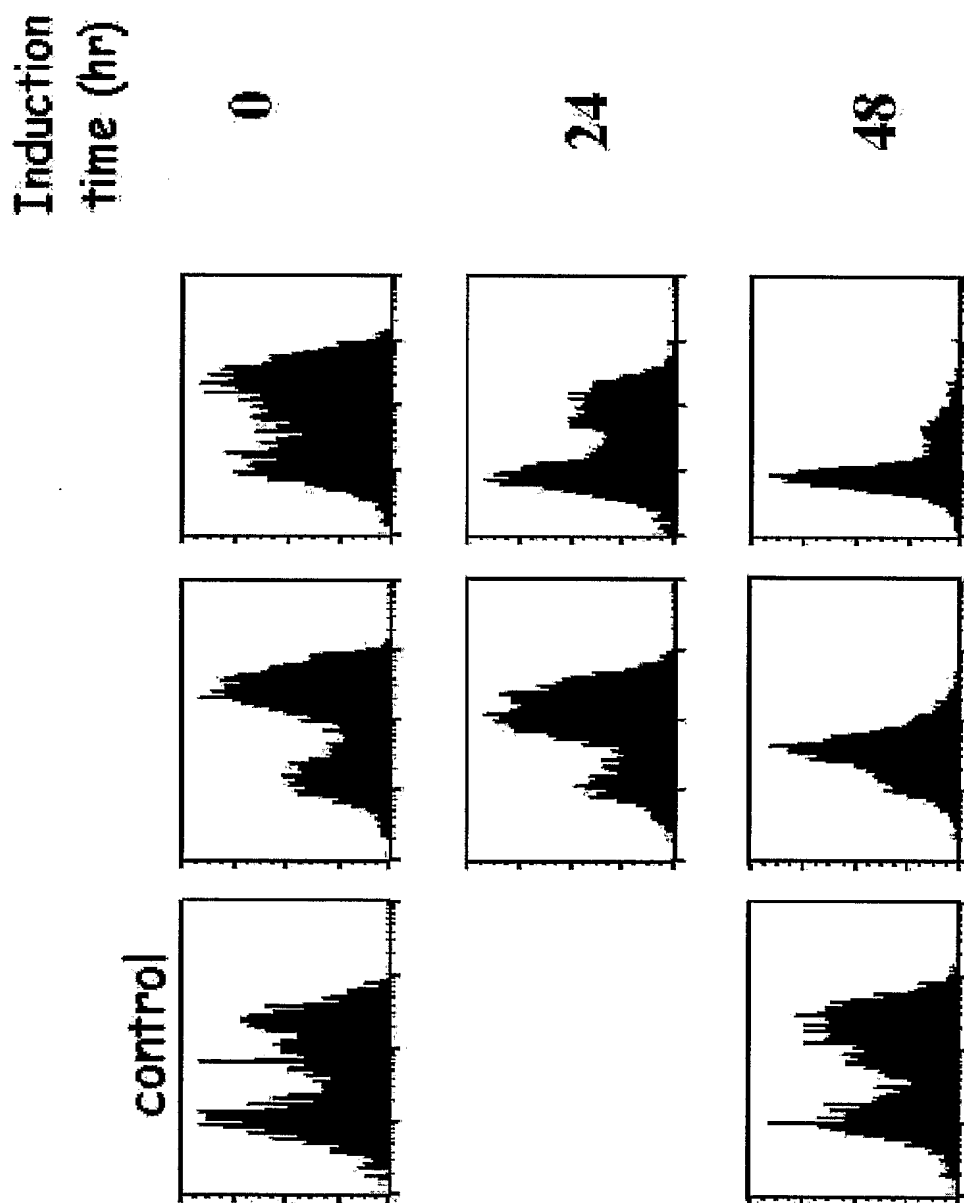


FIG. 12A

Wild-type mouse



TCF-4 KO mouse



FIG. 12B

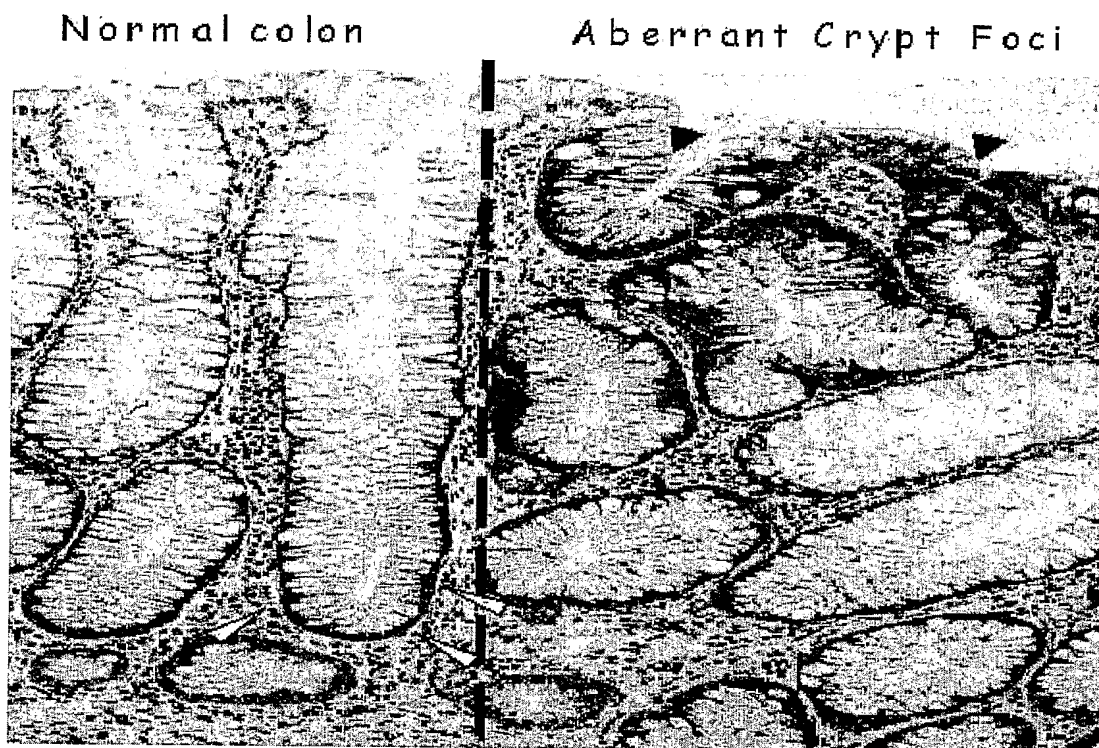


FIG. 13A

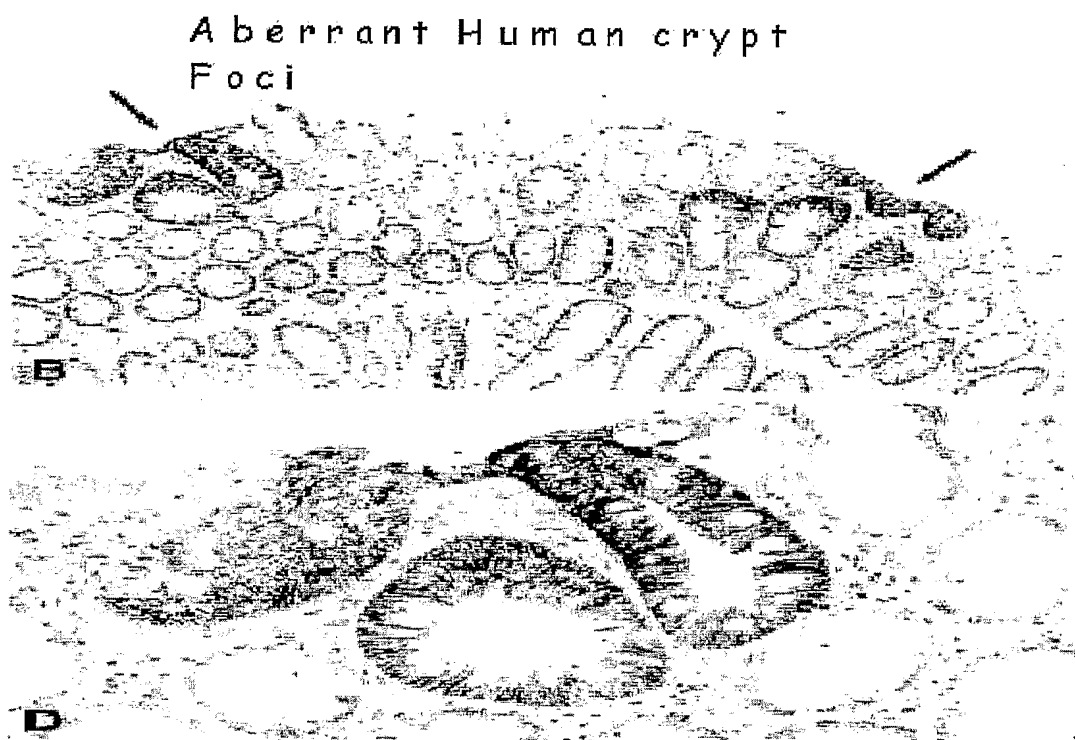


FIG. 13B

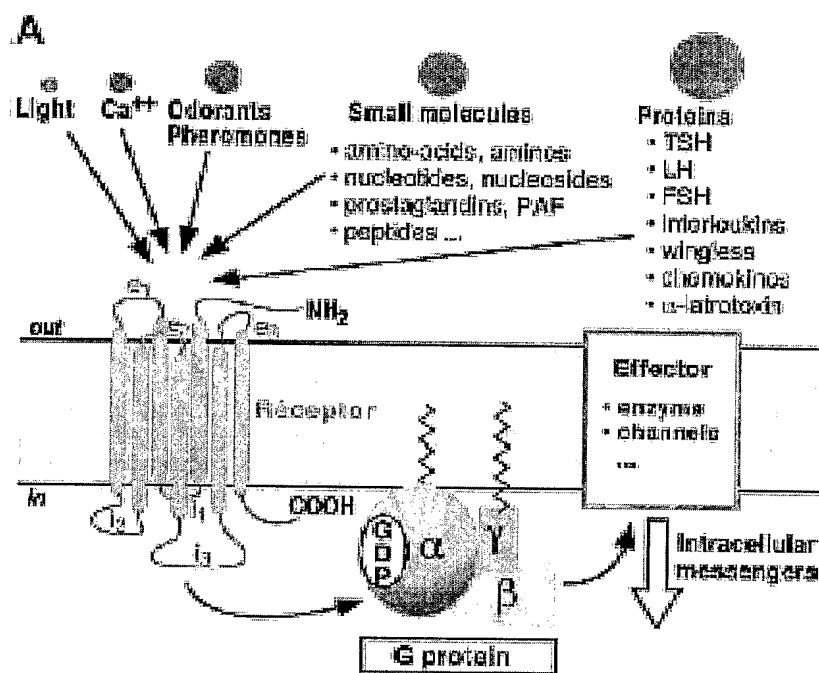


FIG. 14

Figure 1 : Lineup of RGM and RGMR Protein Sequences:

```

humanRGM      --MGRGAG-----RSALGFWP-----TLAFLLCSFPAATS-----PCK
mouseRGM      --MGRGAG-----RSALGLWP-----TLAFLLCSFPAAIS-----PCK
chickenRGM    --MGRGAG-----STALGLFQ-----ILPVFLCIFPPVTS-----PCK
XenopusRGM    MGMGRGAG-----PKALGFFK-----ILTVFLCTFHTVSS-----SCK
HumanRGMR     --MGLRAAPSSAAAAA-AEVEQRRRPGLCP--PPLELLLLLLFSLGLLHAGDCQQPAQCR
mouseRGMR     --MGVRAAPYCAAGPAGAGAEQSRRLWPPTPPPPLLLLLLLSLGLLHAGDCQQPTQCR
               **  * .                :                * .:* :      :      *;

humanRGM      ILKCNSEFWsATS-GSHAPASDDTPEFCAALRSYALCTRRRTARTCRGDLAYHSAVHGIED
mouseRGM      ILKCNSEFWsATSsSGSHAPASDDVPEFCAALRTYALCTRRRTARTCRGDLAYHSAVHGIED
chickenRGM    ILKCNSEFWAATS-GSHHLGAEETPEFCTALRAYAHCTRRRTARTCRGDLAYHSAVHGIDD
XenopusRGM    ILKCTADYLQATSNPHHHTGAEDTVEICTALRTYAHCSRRTARTCRGDLAYHSTVHGIDD
HumanRGMR     IQKCTTDFVSLTSHLNSAVDGFDS-EFCKALRAYAGCTQRTSKACRGNLVYHSAVLGISD
mouseRGMR     IQKCTTDFVALTAHLNSAADGFDS-EFCKALRAYAGCTQRTSKACRGNLVYHSAVLGISD
               * ** .:::  * :          . :  *:* ***:* * :*:*:*:*:*:*.***:* **.*

humanRGM      LMSQHNCsKDGPTSQPRLRTLPPAGDSQERSDSPEICHYEKSfHKHSATPNYTHCGLFGD
mouseRGM      LMSQHNCsKDGPTSQPVRRTLPPAGDSQERSDSPEICHYEKSfHKHSAAPNYTHCGLFGD
chickenRGM    LMVQHNCsKDGPTSQPRLRTLPP-GDSQERSDSPEICHYEKSfHKHSAAPNYTHCGLFGD
XenopusRGM    LMSHHNCsKDGPTSQPVRVRLPP-GDSQERSDSPEICHYEKSfHRPSALPNYTHCGLFGD
HumanRGMR     LMSQRNCsKDGPTSSTNPEVTHDPCNYHSHAGAREHRRGDQ-----NPPSYLFCGLFGD
mouseRGMR     LMSQRNCsKDGPTSSTNPEVTHDPCNYHSHGGVREHGGGDQ-----RPPNYLFCGLFGD
               ** :*:*****... .      : :...  *      ::      *.* .*****

humanRGM      PHLRTFTDRFQTCKVQGAWPLIDNNYLNvQATNTPVLPgSAATATSKLTIIFKNFQECVD
mouseRGM      PHLRTFTDHFQTCKVQGAWPLIDNNYLNvQVTNTPVLPgSAATATSKLTIIFKNFQECVD
chickenRGM    PHLRTFTDTFQTCKVQGAWPLIDNNYLNvQVTNTPVLPgSSATATSKLTIIFKSFQECVE
XenopusRGM    PHLRTFSDTFQTCKIQGAWPLIDNNYLNvQVTNTPVLPgSTATATSKLTIIFKNFQECVD
HumanRGMR     PHLRTFKDNFQTCKVEGAWPLIDNNYLSVQVTNVPVVGSSATATNKITIIFKAHHECTD
mouseRGMR     PHLRTFKDHFQTCKVEGAWPLIDNNYLSVQVTNVPVVGSSATATNKVTIIFKAQHECTD
               *****.* *****:;*****.***.***.***:*****.*:***** :*.:

```

FIG. 15A

humanRGM	QKVYQAEMDELPAAFDGSKNGGDKHGANSKITEKVSQGHVEIQAKYIGTTIVVRQVGR
mouseRGM	QKVYQAEMDELPFAFADGSKNGGDKHGANSKITEKVSQGHVEIQAKYIGTTIVVRQVGR
chickenRGM	QKVYQAEMDELPAAFDGSKNGGDKHGANSKITEKVSQGHVEIQAKYIGTTIVVRQVGR
XenopusRGM	QKVYQAEMDELPAAFDGSKNGGDKSGANSRLRIIEKVSQGHVEIQAKYIGTTIVVRQVGH
HumanRGMR	QKVYQAVTDDLPAAFVDGTTSGGD--SDAKSLRIVERESGHYVEMHARYIGTTVFVRQVGR
mouseRGMR	QKVYQAVTDDLPAAFVDGTTSGGD--GDVKS LHIVEKESGRYVEMHARYIGTTVFVRQLGR
	***** *:*** ** :..*** ..:*** * *: **::*:**::*****:..***:*
humanRGM	YLTFAVRMPEEVVNAVEDWDSQGLYLCLRGCPNQQIDFQAFH-TNAEGTGARRLAAASP
mouseRGM	YLTFAVRMPEEVVNAVEDDRSQGLYLCLRGCPNQQIDFQAFR-ANAE--SPRRPAAASP
chickenRGM	YLTFAVRMPEEVVNAVEDDRSQGLYLCLRGCPNQQIDFQTFRLAQAAGRARRKGFSLP
XenopusRGM	YLTFAVRMPEEVVNAVEDKDNQGLYLCLHGCPCPNQQIDFRNFH-LQAPETGLKRITSASS
HumanRGMR	YLTLAIRMPEDLAMSYEE--SQDLQLCVNGCPLSERIDDGQGVSAILGHSLPRTSLVQA
mouseRGMR	YLTLAIRMPEDLAMSYEE--SQDLQLCVNGCPMSECIDDGQGVSAILGHSLPHTTSVQA
	:*::.. : *: .*. * **:.* ** .: ** : : .
humanRGM	APTAPETFPYETAVAKCKEKLPEVDLYYQACVFDLLTTGDVNFTLAAYYALEDVKMLHSN
mouseRGM	SPVVPETFPYETAVAKCKEKLPEVDLYYQACVFDLLTTGDVNFTLAAYYALEDGKMLHSN
chickenRGM	AP--PEAFTYESATAKCKEKLPEVDLYYQACVFDLLTTGDVNFTLAAYYAFEDVKMLHSN
XenopusRGM	AA----SFTPQTAEAKCKEKLPEVDLYYQACVFDLLTTGDVNFTLAAYYAFEDVKMLHSN
HumanRGMR	WP----GYTLETANTQCHEKMPVKDIYFQSCVFDLLTTGDANFTAAHSALEDVEALHPR
mouseRGMR	WP----GYTLETASTQCHEKMPVKDIYFQSCVFDLLTTGDANFTAAHSALEDVEALHPR
	. :. :.* :*:***:***:***:*****.* ** :*. ** : **..
humanRGM	KDKLHLYERTRDLPGRRAAG-----LPLAPRPLLGALVPLLALLPVFC---
mouseRGM	KDKLHLFERTRELPGAVAAAAAATTFPLAPQILG-TIPLLVLLPVW---
chickenRGM	KDKLHLYERTRALAPGNAAP-----SEHPWALPALWVALLSLSQCWGLLI
XenopusRGM	KNKVHLFERP-----
HumanRGMR	KERWHIFPSSGNGTP-----RGGSDLSVSLGLTCLILIVFL----
mouseRGMR	KERWHIFPSSCG-----GCRDLPVGLGLTCLILIMFL----
	*: *:: .

FIG. 15B

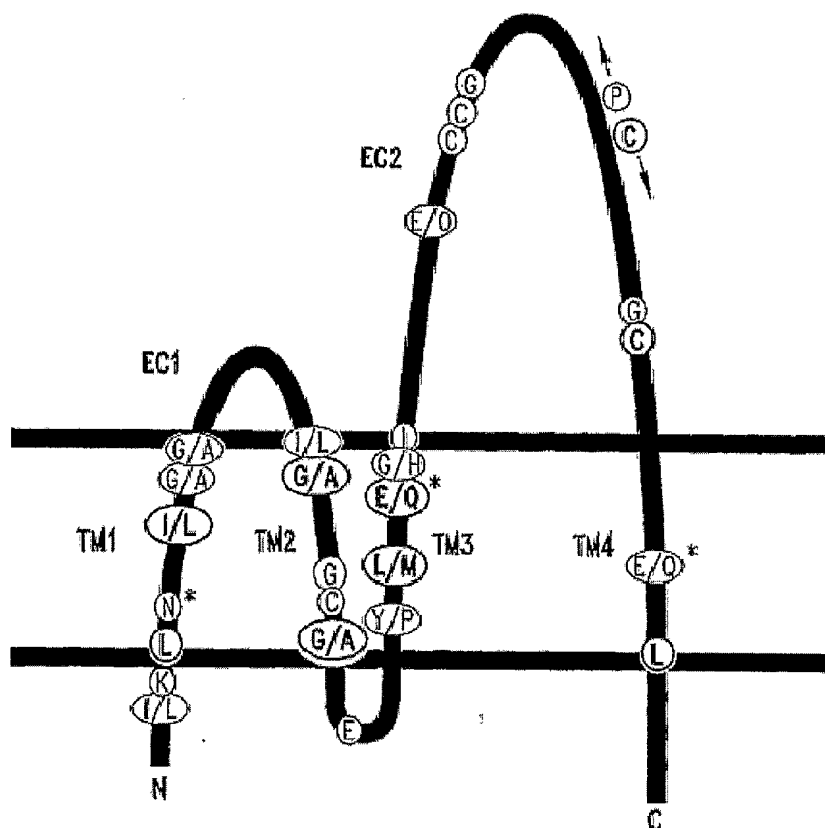


FIG. 16

ATGGACAAGTTTTGGTGGCAGCAGCCTGGGACTCTGCCTCGTGCCGCTGAGCCTGGCGCAGATCGATTGGAATATAACCTGCCG
CTTTGACAGGTGTATCCACGTGGAGAAAAATGGTCGCTACAGCATCTCTCGGACGGAGGCCGCTGACCTCTGCAAGGCTTTCAATA
GCACCTTGCCCACAATGGCCAGATGGAGAAAGCTCTGAGCATCGGATTGAGACCTGCAGGTATGGGTTTCATAGAAGGGCAGCTG
GTGATTCCCGGATCCACCCCACTCCATCTGTGCAGCAAAACACAGGGGTGTACATCCTCACATCCAACACCTCCAGTATGA
CAGATATTGCTTCAATGCTTCAGTCCACCTGAAGAAGATTGTACATCAGTCACAGACCTGCCCAATGCCTTTGATGGACCAATTA
CCATAACTATTGTTAACCGTGATGGCACCCTGATGTCCAGAAAGGAGAATACAGAACGAATCCTGAAGACATCTACCCAGCAAC
CCTACTGATGATGACGTGAGCAGCGGCTCCTCCAGTGAAGGAGCAGCACTTCAGGAGGTTACATCTTTTACACCTTTTCTACTGT
ACACCCCATCCAGACGAAGACAGTCCCTGGATCACCAGCAGCAGACAGAGAATCCCTGCTACCCTTTGATGAGCACTAGTGCTA
CAGCAACTGAGACAGCAACCAAGAGGCAAGAAACCTGGGATTGGTTTTTCATGGTTGTTTCTACCATCAGAGTCAAAGAATCATCTT
CACACAACACACAAATGGCTGGTACGCTCTCAAATACCATCTCAGCAGGCTGGGAGCCAAATGAAGAAAATGAAGATGAAGAGA
CAGACACCTCAGTTTTTCTGGATCAGGCATTGATGATGATGAAGATTTTATCTCCAGCACCATTTCACACACACACCGGCTTTT
ACCACACAAAACAGAACAGGACTGGACCCAGTGAACCAAGCCATTCAAATCCGGAAGTGTACTTCAGACAACCAAGGATG
ACTGATGTAGACAGAAATGGCACCCTGCTTATGAAGGAACTGGAACCCAGAGCAGACCCCTCCCTCATTCACCATGAGCATCA
TGAGGAAGAAGAGACCCCACTTCTACAAGCACAATCCAGGCACTCCTAGTAGTACAACGGAAGAACAGCTACCCAGAAGGAAC
AGTGGTTTGGCAACAGATGGCATGAGGGATATCGCCAAACCCAGAGAAGACTCCCATTCGACAACAGGGACAGCTGAGCCCTCA
GCTCATACCAGCCATCCAATGCAAGGAAGGACAACACCAAGCCAGAGGACAGTTCTCGGACTGATTTCTTCAACCCAACTCTACA
CCCCATGGGACGAGGTCAATCAAGCAGGAAGAAGGATGGATGGACTCCAGTCAATGATATAACGCTTCAGCCTACTGCAATCCAA
ACACAGGTTTGGTGAAGATTGGACAGGACAGGACCTCTTTCAATGACAACGAGCAGAGTAATCTCAGAGCTTCTCTACATCA
CATGAAGGCTTGAAGAAGATAAAGACCATCCAACAACCTCTACTCTGACATCAAGCAATAGGAATGATGTACAGGTGAAGAAG
AGACCCAAATCACTTGAAGGCTCAACTACTTTACTGGAAGGTTATACCTCTCATTACCCACACAGGAAGGAAGCAGGACCTTCA
TCCCAGTGACCTCAGCTAAGACTGGGTCTTTGGAGTTACTGCAGTTACTGTTGGAGATTCCACTCTAATGTCAATCGTTCTCTTA
TCAGGAGACCAAGACACATTCCACCCAGTGGGGGCTCCCATACCCTCATGGATCTGAATCAGATGGACACTCACATGGGAGTCA
AGAAGGTGGAGCAACACAACCTCTGGTCTATAAGGACACCCCAATTCAGAAATGGCTGATCATCTTTGGCATCCCTCTTGGCCT
TGGCTTTGATTCTTGCAGTTTGCATTGCACTCAACAGTCAAGAGGTTGTGGGCAAGAAAAGCTAGTGTATCAACAGTGGCAAT
GGAGCTGTGGAGGACAGAAAGCCAAGTGGACTCAACGGAGAGGCCAGCAAGTCTCAGGAAATGGTGCATTTGGTGAACAGGAGTC
GTCAGAACTCCAGACCAAGTTTATGACAGCTGATGAGACAAAGGAACCTGCAGAAATGTGGACATGAAGATTGGGGTGTACACCTAC
ACCATTAATCTTGAAGAAGAACACCCGTTGAAACATAACCATACAGGAGCTGGGACACTTAACAGATGCAATGTGCTACTGATT
GTTTCATTGCGAATCTTTTTTAGCATAAAATTTCTACTCTTTTTGTTTGTGTTTTGTTTGTGTTTTGTTTGTGTTTTGTTTGTG
AAAACAGCATTTGCTTTCTGAAATTAGGGCCCAATTAATAATCAGCAAGAATTGATCGTCCAGTTCCCACTTGGAGGCTTTTCAT
CCCTCGGGTGTGCTATGGATGGCTTCTAACAACCACTACACATATGATTCTCGATCGCCCAACCTTTCCCCCAGGCTTAAGGACA
TTTCCCAGGGTTAATAGGGCCCTGGTCCCTGGGAGGAAATTTGAATGGGTCCATTTTGGCCTTCCATAGCCTAATCCCTGGGCTATG
CTTTCCACTGAGGTTGGGGGTGGGGTGTACTAGTTACACATCTTCAACAGACCCCTCTAGAAATTTTCAGATGCTTCTGGGAG
ACACCAAGGGTGAAGCTATTTATCTGTAGTAACTATTTATCTGTGTTTTTGAATATTTAAACCTGGATCAGTCCCTTTGATCA
GTATAATTTTTTAAAGTTACTTTGTGACAGGCACAAAGGGTTTAAACTGATTCATAATAAATATCTGACTTCTTCGATCTTCAC
CTTTTGTGCTGTGATTCTTCAGTTTCTAAACAGCACTGTCTGGTCCCTACAATGTATCAGGAAGAGCTGAGAATGGTAAGGAGA
CTCTTCTAAGTCTTCATCTCAGAGACCTGAGTTCCCACTCAGACCACTCAGCCAAATCTCATGGAAGACCAAGGAGGGCAGCAC
TGTTTTGTGTTTTTGTGTTTTTGTGTTTTTGTGTTTTTGTGTTTTTGTGTTTTTGTGTTTTTGTGTTTTTGTGTTTTTGTGTTTT
GGAGCTTCAGCCTCTTTTATGGTTTAAATGGCCACCTGTTCTCTCCCTGTTGAAAGGCTTTGCAAGTCAATTAAGTTTGCATGACCT
GTTATCCCTGGGGCCCTATTTTCATAGAGGCTGGCCCTATTAGTGATTTCACAAAACATATGGAAGTGCCTTTTGTATGCTTTACAA
TAAGAGAAGAAAGCCAATGGAATGAAGAGATTGGCAAGGGGAAGGATGATGCCATGTAGATCCTGTTTGTGATTTTTATGGCTG
TATTTGTAACTTAAACACACCAAGTGTCTGTTGATGCAAGTTGCTATTTTAGGATGAGTTAAGTGCCTGGGAGTCCCTCAAAGG
TTAAAGGGATTCCCATCATTTGGAATCTTATCACCAGATAGGCAAGTTTATGACCAAAACAGAGAGTACTGGCTTTATCCTCTAAC
TCATATTTTCTCCCACTTGGCAAGTCCCTTTGTGGCATTTATTCATCAGTCAGGGGTGCCGATGGTCTAGAACTTCCAAAGGCTG
CTTGTGCATAGAAGCCATTGCATCTATAAAGCAACGGCTCCTGTTAAATGGTATCTCCTTTCTGAGGCTCCTACTAAAAGTCAATTG
TTACCTAACTTATGTGCTTAACAGGCAATGCTTCTCAGACCACAAGCAGAAAGAAGAAAAGGCTCCTACTAAAAGTCAATTG
GGGCTTAGACAGAGTTGATCTGTAGAAATATCTTAAAGGAGAGATGTCAACTTTCTGCACATTTCCAGCCTCTGCTCCTCCTGT
CTACCTTCCCTCCCTCCTCCTCCCTCCACTTCAACCCACATCTTGAAAACTTCTCTTCTCTGTGAACATCATTTGGCCAGA
TCCATTTTTCAGTGGTCTGGATTTCTTTTTATTTTCTTTTCAACTTGAAAGAACTGGACATTAGGCCACTATGTGTTTACTGCTC
ACTAGTGTCAAGTGCTCTTGTGTTTTCCAGAGATTTCCTGGGTCTGCCAGAGGCCAGACAGGCTCAAGCTCTTAACTGA
AAGCAACAAGCCACTCCAGGACAAGGTTCAAATGGTTACAACAGCCTCTACCTGTGCCCCAGGGAGAAAGGGGTAGTGATACA
AGTCTCATAGCCAGAGATGGTTTTCCACTCCTCTAGATATTTCCAAAAGAGGCTGAGACAGGAGTTATTTTCAATTTTTATTT
GGAATTAATACTTTTTTCCCTTTATTAAGTGTGTAGTCCCTCACTTGGATATACCTCTGTTTTACAGATAGAATAAGGGAGGTC
TAGAGCTTCTATTTCTGGCCATTGTCAACGGAGAGCTGGCCAACTTCAACAAACCTTGAACATTTGCTGAAGTTTATGGAAT
AAGATGTATTTCTCACTCCTTGTATCTCAAGGGCTTAACCTGGAAGCAGAGCTTGACTACAGTCAATTTTACCAATGATTTTTCAG
GTGACCTGGGCTAAGTCATTTAACTGGGTCTTTATAAAGTAAAGGCCAACATTTAATTTATTTGCAAGCAACCTAAGAGCTA
AAGATGTAATTTTTCTGCAATTTGTAATCTTTTGTGTCTCTGAAGACTTCCCTTAAATTAGCTCTGAGTGAAAAATCAAAGA
GACAAAAGACATCTCGAATCCATATTTCAAGCCTGGTAGAATTTGGCTTTTCTAGCAGAACCTTTCCAAAAGTTTATATTGAGAT
TCATAACAACACCAAGAATTGATTTTGTAGCCAACATTTCAATACTGTATATCAGAGGAGTAGGAGAGAGGAAACATTTGAC
TTATCTGGAAGAAAGCAAAATGACTTAAGAAATAAGATAACATGGTCCATTACCTTTATGTTATAGATATGCTTTTGTGTAATCA
TTTGTTTTGTAGTTTTCAAGAATAGCCATTGTTCTATTCTGTGCTGTACAATGACCACGTGTTATGTTACTTTTACTTTTTCAGAG
CACACCTTCCCTGTTTGTATATTTTATGATGATCAATAATAATGAGGAAGCATGATATGATATTGCTGAGTTGAAAGC
ACTTATTGAAAAATATTAAGGCTAACATTAAGACTAAGGAAACAGACTCAGA

(SEQ ID No. 1)

FIG. 17A

MDKFWWHAAWGLCLVPLSLAQIDLNITCRFAGVFHVEKNGRYSISRTEAADLCKAFNSTLPTMAQMEKALSIGFETCR
YGFIEGHVVIPRIHPNSICAANNTGVYILTSNTSQYDTCFNASAPPEEDCTSVTDLPNAFDGPITITIVNRDGTTRYVQKGE
YRTNPEDIYPSNPTDDDDVSSGSSERSSTSGGYIFYTFSTVHPIPEDDSPWITDSTDRIPATTLMSTSATATETATKRQETW
DWFSWLFLPSESKNHLHTTTQMAGTSSNTISAGWEPNEENEDERDRHLSFSGSGIDDDDEDFFISSTISTTPRAFDHTKQNQ
DWTQWNPSHSNPEVLLQTTTRMTDVDRNGTTAYEGNWNPEAHPPLIHHEHHHEEEETPHSTSTIQATPSSTTEETATQKE
QWFGNRWHEGYRQTPREDSSHSTTGTAASAHTSHPMQGRTPSPEDSSWTDFFNPISHPMGRGHQAGRMDMDSSHSI
TLQPTANPNTGLVEDLDRTGPLSMTTQQSNSQSFSSTHEGLEEDKDHPPTSTLTSSNRNDVTGGRRDPNHSEGSTLLEG
YTSHYPHTKESRTFIPVTSAKTGSFGVTA VTVGDSNSNVNRLSGDQDTFHPSGGSHTHGSESDGHSHGSQEGGANTT
SGPIRTPQIPEWLIIILASLLALILAVCIAVNSRRRCGQKKKLVINSGNGAVEDRKPSGLNGEASKSQEMVHLVNKESSE
TPDQFMTADETRNLQNVDKIGV*

(SEQ ID No. 2)

FIG. 17B

ATGGACAAGTTTTGGTGGCACGCAGCCTGGGGACTCTGCCTCGTGCCGCTGAGCCTGGCGCAGATCGATTGAATATAACCTGCCG
CTTTGCAGGTGTATTCACGTGGAGAAAAATGGTCGCTACAGCATCTCTCGGACGGAGGCCGCTGACCTCTGCAAGGCTTTCAATA
GCACCTTGCCCAACAATGGCCAGATGGAGAAAGCTCTGAGCATCGGATTTGAGACCTGCAGGTATGGGTTTCATAGAAGGGCATGTG
GTGATTCCCCGGATCCACCCCACTCCATCTGTGCAGCAACAACACAGGGGTGTACATCCTCACATACAACACCTCCCAGTATGA
CACATATTGCTTCAATGCTTCAGCTCCACCTGAAGAAGATTGTACATCAGTCACAGACCTGCCCAATGCCTTTGATGGACCAATTA
CCATAACTATTGTAAACCGTGATGGCACCCGCTATGTCCAGAAAAGGAGAATACAGAACGAATCCTGAAGACATCTACCCAGCAAC
CCTACTGATGATGACGTGAGCAGCGGCTCCTCCAGTGAAGGAGCAGCACTTCAGGAGGTTACATCTTTTACACCTTTTCTACTGT
ACACCCCATCCCAGACGAAGACAGTCCCTGGATCACCGACAGCACAGACAGAATCCCTCGTACCAATATGGACTCCAGTCATAGTA
CAACGCTTCAGCCTACTGCAAATCCAAACACAGGTTTGGTGAAGATTTGGACAGGACAGGACCTTTTCAATGACAACGCAGCAG
AGTAATTCTCAGAGCTTCTCTACATCACATGAAGGCTTGAAGAAGATAAAGACCATCCAACAACCTTCTACTCTGACATCAAGCAA
TAGGAATGATGTCACAGGTGGAAGAAGAGACCCAAATCATTTCTGAAGGCTCAACTCATTACTGGAAGGTATACCTCTCATTACC
CACACACGAAGAAAGCAGGACCTTCATCCCAGTGACCTCAGCTAAGACTGGGTCCTTTGGAGTTACTGCAGTTACTGTTGGAGAT
TCCAACCTCAATGTCAATCGTTCCCTTATCAGGAGACCAAGACACATTCCACCCAGTGGGGGGTCCCATACCACTCATGGATCTGA
ATCAGATGGACACTCACATGGGAGTCAAGAAGGTGGAGCAACACAACCTCTGGTCCTATAAGGACACCCCAATTCAGAATGGC
TGATCATCTTGGCATCCCTCTTGGCCTTGGCTTTGATTCTTGCAGTTTGCATTGCAGTCAACAGTCGAAGAAGGTGTTGGCAGAAG
AAAAAGCTAGTGATCAACAGTGGCAATGGAGCTGTGGAGGACAGAAAGCCAAGTGGACTCAACGGAGAGGCCAGCAAGTCTCAGGA
AATGGTGCATTGTGTGAACAAGGAGTCGTCAGAACTCCAGACCAGTTTATGACAGCTGATGAGACAAGGAACCTGCAGAATGTGG
ACATGAAGATTGGGGTGTAA

FIG. 18A

MDKFWHAAWGLCLVPLSLAQIDLNITCRFAGVFHVEKNGRYSISRTEAADLCKAFNSTLPTMAQMEKALSIGFETCRYGFIEGHV
VIPRIHPNSICAANNTGVYILTYNTSQYDTYCFNASAPPEEDCTSVTDLPNAFDGPITITIVNRDGTTRYVQKGEYRTNPEDIYPSN
PTDDDVSSGSSSERSSTSGGYIFYTFSTVHPIPEDSPWITDSTDRIPRTNMDSHSTTLQPTANPNTGLVEDLDRTGPLSMTTQQ
SNSQSFSSTSHGLEEDKDHPTTSTLTSSNRNDVTGGRDPNHSEGSTHLLLEGYTSHPHTKESRTFIPVTSAKTGSFGVTAFTVGD
SNSNVNRLSGDQDTFHPSGGSHTHGSESDGHSQEGGANTTSGPIRTPQIPEWLIILASLLALALILAVCIAVNSRRRCGQK
KKLVINSGNGAVEDRKPSGLNGEASKSQEMVHLVNKESSETPDQFMTADETRNLQNVDMKIGV

FIG. 18B

CTTTGATGAGCACTAGTGCTACAGCAACTGAGACAGCAACCAAGAGGCAAGAAGCCTGGGATTGGTTTTCATGGTTGTTTCTACCA
TCAGAGTCAAAGAATCATCTTCACACAACAACACAAATGGCTG

FIG. 19A

GTACGTCTTCAAATACCATCTCAGCAGGCTGGGAGCCAAATGAAGAAAATGAAGATGAAAGAGACAGACACCTCAGTTTTTCTGGA
TCAGGCATTGATGATGATGAAGATTTTATCTCCAGCACCA

FIG. 19B

TTTCAACCACACCACGGGCCTTTGACCACACAAAACAGAACAGGACTGGACCCAGTGGAACCCAAGCCATTCAAATCCGGAAGTG
CTACTTCAGACAACCACAAGGATGACTG

FIG. 19C

ATGTAGACAGAAATGGCACCCTGCTTATGAAGGAACTGGAACCCAGAACACACCCCTCCCCTCATTCACCATGAGCATCATGAG
GAAGAAGAGACCCACATTCTACAAGCACAA

FIG. 19D

TCCAGGCAACTCCTAGTAGTACAACGGAAGAAACAGCTACCCAGAAGGAACAGTGGTTTGGCAACAGATGGCATGAGGGATATCGC
CAAACACCCAGAGAAGACTCCCATTCGACAACAGGGACAGCTG

FIG. 19E

CAGCCTCAGCTCATACCAGCCATCCAATGCAAGGAAGGACAACACCAAGCCCAGAGGACAGTTCTGGACTGATTTCTTCAACCCA
ATCTCACACCCCATGGGACGAGGTCATCAAGCAGGAAGAAGGATGG

FIG. 19F

ATATGGACTCCAGTCATAGTACAACGCTTCAGCCTACTGCAAATCCAACACAGGTTTGGTGGAAAATTTGGACAGGACAGGACCT
CTTTCAATGACAACGC

FIG. 19G

AGCAGAGTAATTCTCAGAGCTTCTTACATCACATGAAGGCTTGGAAGAAGATAAAGACCATCCAACAACCTTCTACTCTGACATCA
AGCA

FIG. 19H

ATAGGAATGATGTCACAGGTGGAAGAAGAGACCCAAATCATTCTGAAGGCTCAACTACTTTACTGGAAGGTTATACCTCTCATTAC
CCACACACGAAGGAAAGCAGGACCTTCATCCCAGTGACCTCAGCTAAGACTGGGTCCTTTGGAGTTACTGCAGTTACTGTTGGAGA
TTCCAACCTAATGTCAATCGTTCCTTATCAG

FIG. 19I

Sequences of GPR49**A) Nucleic sequence GPR49 mRNA sequence:**

>gi|4504378|ref|NM_003667.1| Homo sapiens G protein-coupled receptor 49 (GPR49), mRNA
ATGGACACCTCCCGGCTCGGTGTGCTCCTGTCTTGGCTGTGCTGCTGCAGCTGGCGACCGGGGGGAGCTC
TCCCAGGCTCTGGTGTGTTGCTGAGGGGCTGCCCCACACACTGTCATTGCGAGCCCGACGGCAGGATGTTGC
TCAGGGTGGACTGCTCCGACCTGGGGCTCTCGGAGCTGCCTTCCAACCTCAGCGTCTTCACCTCCTACCTA
GACCTCAGTATGAACAACATCAGTCAGCTGCTCCCGAATCCCCTGCCAGTCTCCGCTTCCCTGGAGGAGTT
ACGTCTTGC GGAAACGCTCTGACATACATTCCCAAGGGAGCATTCACTGGCCTTTACAGTCTTAAAGTTC
TTATGCTGCAGAATAATCAGCTAAGACACGTACCCACAGAAGCTCTGCAGAATTTGCCGAAGCCTTCAATCC
CTGCGTCTGGATGCTAACCACATCAGCTATGTGCCCCAAGCTGTTTCAGTGGCCTGCATTCCCCTGAGGCA
CCTGTGGCTGGATGACAATGCGTTAACAGAAATCCCCGTCCAGGCTTTTAGAAGTTTATCGGCATTGCAAG
CCATGACCTTGGCCCTGAACAAAATACACCACATACCAGACTATGCCTTTGGAAACCTCTCCAGCTTGGTA
GTTCTACATCTCCATAACAATAGAATCCACTCCCTGGGAAAGAAATGCTTTGATGGGCTCCACAGCCTAGA
GACTTTAGATTTAAATTACAATAACCTTGATGAATCCCCACTGCAATTAGGACACTCTCCAACCTTAAAG
AACTAGGATTTTCATAGCAACAATATCAGGTCGATACCTGAGAAAGCATTGTAGGCAACCCCTTCTCTTATT
ACAATACATTTCTATGACAATCCCATCCAATTTGTTGGGAGATCTGCTTTTCAACATTTACCTGAACCTAAG
AACACTGACTCTGAATGGTGCCTCACAATAACTGAATTTCTGATTTAACTGGAACCTGCAACCTGGAGA
GTCTGACTTTAACTGGAGCACAGATCTCATCTCTTCTCCTCAACCGTCTGCAATCAGTTACCTAATCTCCAA
GTGCTAGATCTGCTTTACAACCTATAGAAAGATTTACCCAGTTTTTTCAGTCTGCCAAAAGCTTCAGAAAAT
TGACCTAAGACATAATGAAATCTACGAAATTAAGTTGACACTTTCCAGCAGTTGCTTAGCCTCCGATCGC
TGAATTTGGCTTGGAAACAAATTGCTATTATTACCCCCAATGCATTTTCCACTTTGCCATCCCTAATAAAG
CTGGACCTATCGTCCAACCTCCTGTCGTCTTTTCTCTATACTGGGTTACATGGTTTAACTCACTTAAATTT
AACAGGAAATCATGCCTTACAGAGCTTGATATCATCTGAAAACCTTTCCAGAACTCAAGGTTATAGAAATGC
CTTATGCTTACCAGTGTCTGTCATTTGGAGTGTGTGAGAATGCCTATAAGATTTCTAATCAATGGAATAAA
GGTGACAACAGCAGTATGGACGACCTTCATAAGAAAGATGCTGGAATGTTTCAGGCTCAAGATGAACGTGA
CCTTGAAGATTTCCCTGCTTGACTTTGAGGAAGACCTGAAAGCCCTTCATTTCAGTGCAGTGTTCACCTTCCC
CAGGCCCTTCAAACCTGTGAACACCTGCTTGATGGCTGGCTGATCAGAATTTGGAGTGTGGACCATAGCA
GTTCTGGCACTTACTTGTAAATGCTTTGGTGACTTCAACAGTTTTTCAGATCCCCCTGTACATTTCCCCCAT
TAACTGTTAATTGGGGTCATCGCAGCAGTGAACATGCTACGCGGAGTCTCCAGTGCCGTGTGGCTGGTG
TGGATGCGTTCACTTTTGGCAGCTTTGCACGACATGGTGCCTGGTGGGAGAATGGGGTTGGTTGCCATGTC
ATTGGTTTTTTTGCTTCAATCATCTGTTTTCTGCTTACTCTGGCAGCCCTGGAGCGTGG
GTTCTCTGTGAAATATTCTGCAAAATTTGAAACGAAAGCTCCATTTTCTAGCCTGAAAGTAATCATTTTGC
TCTGTGCCCTGCTGGCCTTGACCATGGCCGAGTTCCCCTGCTGGGTGGCAGCAAGTATGGCGCTCCCTCT
CTCTGCCCTGCCCTTTGCCCTTTGGGGAGCCCAGCACCATGGGCTACATGGTCTGCTCTCATCTTGCTCAATTC
CCTTTGCTTCCCTCATGATGACCATTGCCCTACACCAAGCTCTACTGCAATTTGGACAAGGGAGACCTGGAGA
ATATTTGGGACTGCTCTATGGTAAAACACATTGCCCTGTTGCTCTTCAACCAACTGCATCCTAACTGCCCT
GTGGCTTTCTTGCTCTTCTCCTCTTTAATAAACCTTACATTTATCAGTCTGAAGTAATTAAGTTTATCCT
TCTGGTGGTAGTCCCACTTCCCTGCATGTCTCAATCCCCTTCTCTACATCTTGTTCATCCTCACTTTAAGG
AGGATCTGGTGGCTGAGCCTGAGAAAGCAAACCTACGTCTGGACAAGATCAAAACACCCAAGCTTGATGTCAATT
AACTCTGATGATGTCGAAAAACAGTCCTGTGACTCAACTCAAGCCTTGGAACCTTTACCAGCTCCAGCAT
CACTTATGACCTGCCTCCAGTTCCGTGCCATCACCAGCTTATCCAGTGACTGAGAGCTGCCATCTTTCTCT
CTGTGGCATTGTCCCATGTCTCTAA (SEQ ID No.3)

FIG. 20A

B) Proteic sequence

>gi|4504379|ref|NP_003658.1| (NM_003667) G protein-coupled receptor 49; G protein-coupled receptor 67; orphan G protein-coupled receptor HG38 [Homo sapiens]

MDTSRLGVLLSLPVLLQLATGGSSPRSGVLLRGCPHCHCEPDGRMLLRVDCSDLGLSELPSNLSVFTS
YLDLSMNNISQLLPNPLPSIRFLEELRLAGNALTYIPKGAFTGLYSLKVLMLQNNQLRHVPTEALQNL
SLQSLRLDANHISYVPPSCFSGLHSLRHLWLDDNALTEIPVQAFRSLSALQAMTLALNKIHHIPDYAFG
NLSSLVVLHLHNNRIHSLGKKCFDGLHSLETDLNYYNNLDEFPTAIRTLNLKELGFHSNNIRSIPKA
FVGNPSLITIHFYDNPIQFVGRSAFQHLPELRTLTNGASQITEFPDLTGTLNLESLTLTGAQISSLPQ
TVCNQLPNLQVLDLSYNLLEDLPSFSVCQKLQKIDLRHNEIYEIKVDTFQQLLSLRSNLAWNKIAIIH
PNAFSTLPSLIKLDLSSNLLSSFPITGLHGLTHLKLGTGNHALQSLISSENFPELKVIEMPYAYQCCAFG
VCENAYKISNQWNKGDNSMDDLHKKDAGMFQAQDERDLEDFLDFFEDLKALHSVQCSPSPGPFKPCE
HLLDGWLIRIGVWTIAVLALTCNALVTSTVFRSPLYISPIKLLIGVIAAVNMLTGVSSAVLAGVDAFTF
GSFARHGAWWENGVGCHVIGFLSIFASESSVFLTLAALERGFSVKYSAKFETKAPFSSLKVIILLCAL
LALTMAAVPLLGGSKYGASPLCLPLPFGEPTMGYVALILLNSLCFLMMTIAYTKLYCNLDKGDLENI
WDCSMVKHIALLLFTNCILNCPVAFLSFSSLINLTFISPEVIKFILLVVVPLPACLNPLLYILFNPHFK
EDLVSLRKQTYVWTRSKHPSLMSINSDDVEKQSCDSTQALVTFTSSSITYDLPPSSVPSPAYPVTESCH
LSSVAFVPCL (SEQ ID No.4)

FIG. 20B

EPHB4 sequence:

A) Nucleic sequence

>gi|17975769|ref|NM_004444.2| Homo sapiens EphB4 (EPHB4), mRNA

CGTCCACCCGCCCAGGGAGAGTCAGACCTGGGGGGGCGAGGGCCCCCAAACCTCAGT
TCGGATCCTACCCGAGTGAGGCGGCGCCATGGAGCTCCGGGTGCTGCTCTGCTGGGC
TTCGTTGGCCGCAGCTTTGGAAGAGACCCTGCTGAACACAAAATTGGAAACTGCTGA
TCTGAAGTGGGTGACATTCCCTCAGGTGGACGGGCAGTGGGAGGAACTGAGCGGCCT
GGATGAGGAACAGCACAGCGTGCGCACCTACGAAGTGTGTGAAGTGCAGCGTGCCCC
GGGCCAGGCCCACTGGCTTCGCACAGGTTGGGTCCACGGCGGGGCGCCGTCCACGT
GTACGCCACGCTGCGCTTCACCATGCTCGAGTGCCTGTCCCTGCCTCGGGCTGGGCG
CTCCTGCAAGGAGACCTTCACCGTCTTCTACTATGAGAGCGATGCGGACACGGCCAC
GGCCCTCACGCCAGCCTGGATGGAGAACCCTACATCAAGGTGGACACGGTGGCCGC
GGAGCATCTCACCCGGAAGCGCCCTGGGGCCGAGGCCACCGGGAAGGTGAATGTCAA
GACGCTGCGTCTGGGACCGCTCAGCAAGGCTGGCTTCTACCTGGCCTTCCAGGACCA
GGGTGCCTGCATGGCCCTGCTATCCCTGCACCTCTTCTACAAAAAGTGCGCCAGCT
GACTGTGAACCTGACTCGATTCCCGGAGACTGTGCCTCGGGAGCTGGTTGTGCCCGT
GGCCGCTAGCTGCGTGGTGGATGCCGTCCCCGCCCTGGCCCCAGCCCCAGCCTCTA
CTGCCGTGAGGATGGCCAGTGGGCCGAACAGCCGGTCACGGGCTGCAGCTGTGCTCC
GGGGTTTCGAGGCAGCTGAGGGGAACACCAAGTGCCGAGCCTGTGCCCAGGGCACCTT
CAAGCCCCTGTCAGGAGAAGGGTCCTGCCAGCCATGCCAGCCAATAGCCACTCTAA
CACCATTGGATCAGCCGTCTGCCAGTGCCGCGTCGGGTACTTCCGGGCACGCACAGA
CCCCCGGGGTGCACCCTGCACCACCCCTCCTTCGGCTCCGCGGAGCGTGGTTTCCCG
CCTGAACGGCTCCTCCCTGCACCTGGAATGGAGTGCCCCCTGGAGTCTGGTGGCCG
AGAGGACCTCACCTACGCCCTCCGCTGCCGGGAGTGCCGACCCGGAGGCTCCTGTGC
GCCCTGCGGGGGAGACCTGACTTTTGACCCCGGCCCCCGGGACCTGGTGGAGCCCTG
GGTGGTGGTTTCGAGGGCTACGTCCGGACTTCACCTATACCTTTGAGGTCACTGCATT
GAACGGGGTATCCTCCTTAGCCACGGGGCCCGTCCCATTGAGCCTGTCAATGTCAC
CACTGACCGAGAGGTACCTCCTGCAGTGTCTGACATCCGGGTGACGCGGTCTCTACC
CAGCAGCTTGAGCCTGGCCTGGGCTGTTCCCCGGGCACCCAGTGGGGCGTGGCTGGA
CTACGAGGTCAAATACCATGAGAAGGGCGCCGAGGGTCCCAGCAGCGTGCGGTTCCT
GAAGACGTCAGAAAACCGGGCAGAGCTGCGGGGGCTGAAGCGGGGAGCCAGCTACCT
GGTGCAGGTACGGGCGCGCTCTGAGGCCGGCTACGGGCCCTTCGGCCAGGAACATCA
CAGCCAGACCCAACCTGGATGAGAGCGAGGGCTGGCGGGAGCAGCTGGCCCTGATTGC
GGGCACGGCAGTCGTGGGTGTGGTCTGGTCTGGTGGTCAATTGTGGTCGCAGTTCT
CTGCCTCAGGAAGCAGAGCAATGGGAGAGAAGCAGAATATTCGGACAAACACGGACA
GTATCTCATCGGACATGGTACTAAGGTCTACATCGACCCCTTCACTTATGAAGACCC
TAATGAGGCTGTGAGGGAATTTGCAAAAGAGATCGATGTCTCCTACGTCAAGATTGA
AGAGGTGATTGGTGCAGGTGAGTTTGGCGAGGTGTGCCGGGGGCGGCTCAAGGCCCC
AGGGAAGAAGGAGAGCTGTGTGGCAATCAAGACCCTGAAGGGTGGCTACACGGAGCG
GCAGCGGCGTGAGTTTCTGAGCGAGGCCTCCATCATGGGCCAGTTCGAGCACCCCAA
TATCATCCGCTGGAGGGCGTGGTACCAACAGCATGCCCGTCATGATTCTCACAGA
GTTTCATGGAGAACGGCGCCCTGGACTCCTTCTGCGGCTAAACGACGGACAGTTTAC
AGTCATCCAGCTCGTGGGCATGCTGCGGGGCATCGCCTCGGGCATGCGGTACCTTGC
CGAGATGAGCTACGTCCACCGAGACCTGGCTGCTCGCAACATCCTAGTCAACAGCAA

FIG. 21A

CCTCGTCTGCAAAGTGTCTGACTTTGGCCTTTCCCGATTCTTGAGGAGAACTCTTC
CGATCCCACCTACACGAGCTCCCTGGGAGGAAAGATTCCCATCCGATGGACTGCCCC
GGAGGCCATTGCCTTCCGGAAGTTCACTTCCGCCAGTGATGCCTGGAGTTACGGGAT
TGTGATGTGGGAGGTGATGTCATTTGGGGAGAGGCCGTACTGGGACATGAGCAATCA
GGACGTGATCAATGCCATTGAACAGGACTACCGGCTGCCCCCGCCCCCAGACTGTCC
CACCTCCCTCCACCAGCTCATGCTGGACTGTTGGCAGAAAGACCGGAATGCCCGGCC
CCGCTTCCCCCAGGTGGTCAGCGCCCTGGACAAGATGATCCGGAACCCCGCCAGCCT
CAAAATCGTGGCCCCGGGAGAATGGCGGGGGCCTCACACCCTCTCCTGGACCAGCGGCA
GCCTCACTACTCAGCTTTTGGCTCTGTGGGCGAGTGGCTTCGGGCCATCAAAATGGG
AAGATACGAAGAAAGTTTCGCAGCCGCTGGCTTTGGCTCCTTCGAGCTGGTCAGCCA
GATCTCTGCTGAGGACCTGCTCCGAATCGGAGTCACTCTGGCGGGACACCAGAAGAA
AATCTTGGCCAGTGTCAGCACATGAAGTCCCAGGCCAAGCCGGGACCCCGGGTGGG
ACAGGAGGACCGGCCCGCAGTACTGACCTGCAGGAACTCCCCACCCAGGGACACC
GCCTCCCCATTTTCCGGGGCAGAGTGGGGACTCACAGAGGCCCCCAGCCCTGTGCCC
CGCTGGATTGCACTTTGAGCCCGTGGGGTGAGGAGTTGGCAATTTGGAGAGACAGGA
TTTGGGGGTTCTGCCATAATAGGAGGGGAAAATCACCCCCCAGCCACCTCGGGGAAC
TCCAGACCAAGGGTGAGGGCGCCTTTCCCTCAGGACTGGGTGTGACCAGAGGAAAAG
GAAGTGCCCCAACATCTCCCAGCCTCCCCAGGTGCCCCCTCACCTTGATGGGTGCGT
TCCCGCAGACCAAGAGAGTGTGACTCCCTTGCCAGCTCCAGAGTGGGGGGGCTGTC
CCAGGGGGCAAGAAGGGGTGTCAGGGCCCAGTGACAAAATCATTTGGGGTTTGTAGTC
CCAATTGCTGCTGTCACCACCAAACCTCAATCATTTTTTTCCCTTGTAATGCCCT
CCCCCAGCTGCTGCCTTCATATTGAAGTTTTTTGAGTTTTGTTTTTGGTCTTAATTT
TTCTCCCCGTTCCTTTTTTGTTCCTCGTTTTTGTCTTCTACCGTCCTTGTCATAAC
TTTGTGTTGGAGGGAACCTGTTTCACTATGGCCTCCTTTGCCCAAGTTGAAACAGGG
GCCCCATCATCATGTCTGTTTCCAGAACAGTGCCCTTGGTCATCCCACATCCCCGGACC
CCGCTTGGGACCCCCAAGCTGTGTCCTATGAAGGGGTGTGGGGTGAGGTAGTGAAAA
GGGCGGTAGTTGGTGGTGGAACCCAGAAACGGACGCCGGTGCTTGGAGGGGTTCTTA
AATTATATTTAAAAAAGTAACTTTTTGTATAAATAAAAGAAAATGGGACGTGTCCCA
GCTCCAGGGT (SEQ ID No.5)

FIG. 21B

B) Proteic sequence

>gi|17975770|ref|NP_004435.2| (NM_004444) ephrin receptor EphB4 precursor. Ephrin receptor EphB4 (hepatoma transmembrane kinase); Tyroll; ephrin receptor EphB4; hepatoma transmembrane kinase [Homo sapiens]

MELRVLLCWASLAAALEETLLNTKLETADLKWVTFPQVDGQWEELSGLDEEQHSVRTYEVCEVQRAPGQAH
WLRTGWVPRRGAVHVYATLRFMTMLECLSLPRAGRSCKETFTVFYYESDADTATALTPAWMENPYIKVDTVA
AEHLTRKRPGAEATGKVNKTLRLGPLSKAGFYLAQDQACMALLSLHLFYKKCAQLTVNLTRFPETVPR
ELVVPVAGSCVVDVAVPAPGPSPLYCRELDGQWAEQPVGTGCSCAPGFEEAEGNTKCRACAQGTFFKPLSGEGS
CQPCPANSHSNTIGSAVCQCRVGYFRARTDPRGAPCTTPPSAPRSVVSRLNGSSLHLEWSAPLES GGREDL
TYALRCRECRPGGSCAPCGDLTFDPGPRDLVEPWVVVRGLRPDFTYTFEVTALNGVSSLATGVPVFPFEPVN
VTTDREVPPAVSDIRVTRSSPSSLSLAWAVPRAPSGAWLDYEVKYHEKGAEGPSSVRFLKTSENRAELRGL
KRGASYLVQVRARSEAGYGPFQGEHHSQTQLDESEGWREQLALIAGTAVVGVLVLVIVVAVLCLRKQSN
GREAEYS DKHGQYLIGHGTKVYIDPFTYEDPNEAVREFAKEIDVSYVKIEEVIGAGEFGEVCRGRLKAPGK
KESCVAIKTLKGGYTERQRREFLSEASIMGQFEHPNIIIRLEGVVTNSMPVMILTEFMENGALDSFLRLNDG
QFTVIQLVGMLRGIASGMRYLAEMSYVHRDLAARNILVNSNLVCKVSDFGLSRFLEENSSDPTYTSSLGK
IPIRWTAPEAIAFRKFTSASDAWSYGIVMWEVMSFGERPYWDMSNQDVINAIEQDYRLPPPPDCPTSLHQL
MLDCWQKDRNARPRFPQVVSALDKMIRNPASLKIVARENGGASHPLLDQRQPHYSAFGSVGEWLRAIKMGR
YEESFAAAGFGSFELVSQISAEDLLRIGVTLAGHQKILASVQHMKSQAKPGTPGGTGGPAPQY (SEQ
ID No.6)

FIG. 21C

GPX2 Sequence**A) Nucleic sequence**

>gi|4504102|ref|NM_002083.1| Homo sapiens glutathione peroxidase 2 (gastrointestinal) (GPX2), mRNA

CGGCCTCTCTGCGGGGCTCACTCTGCGCTTCACCATGGCTTTTCATTGCCAAGTCCTT
CTATGACCTCAGTGCCATCAGCCTGGATGGGGAGAAGGTAGATTTCAATACGTTCCG
GGGCAGGGCCGTGCTGATTGAGAATGTGGCTTCGCTCTGAGGCACAACCACCCGGGA
CTTCACCCAGCTCAACGAGCTGCAATGCCGCTTTCCCAGGCGCCTGGTGGTCCTTGG
CTTCCCTTGCAACCAATTTGGACATCAGGAGAACTGTCAGAATGAGGAGATCCTGAA
CAGTCTCAAGTATGTCCGTCCTGGGGGTGGATAACCAGCCACCTTCACCCTTGTCCA
AAAATGTGAGGTGAATGGGCAGAACGAGCATCCTGTCTTCGCCTACCTGAAGGACAA
GCTCCCCTACCCTTATGATGACCCATTTTCCCTCATGACCGATCCCAAGCTCATCAT
TTGGAGCCCTGTGCGCCGCTCAGATGTGGCCTGGAACCTTGAGAAGTTCCTCATAGG
GCCGGAGGGAGAGCCCTTCCGACGCTACAGCCGCACCTTCCCAACCATCAACATTGA
GCCTGACATCAAGCGCCTCCTTAAAGTTGCCATATAGATGTGAACTGCTCAACACAC
AGATCTCCTACTCCATCCAGTCCTGAGGAGCCTTAGGATGCAGCATGCCTTCAGGAG
ACACTGCTGGACCTCAGCATTCCTTGATATCAGTCCCCTTCACTGCAGAGCCTTGC
CTTTCCCCTCTGCCTGTTTCCTTTTCCCTCTCCAACCCTCTGGTTGGTGATTCAACT
TGGGCTCCAAGACTTGGGTAAGCTCTGGGCCTTCACAGAATGATGGCACCTTCCTAA
ACCCTCATGGGTGGTGTCTGAGAGGCGTGAAGGGCCTGGAGCCACTCTGCTAGAAGA
GACCAATAAAGGGCAGGTGTGGAAACGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA (SEQ ID No.7)

FIG. 22A**B) GPX2 Protein Sequence:**

>gi|4504103|ref|NP_002074.1| gastrointestinal glutathione peroxidase 2 [Homo sapiens]

MAFIAKSFYDLSAISLDGEKVDFNTFRGRAVLIENVASLXGTTTRDFTQLNELQCRF
PRRLVVLGFPCNQFGHQENCQNEEILNSLKYVRPGGYQPTFTLVQKCEVNGQNEHP
VFAYLKDKLPYPYDDPFSLMTDPKLI IWSPVRRSDVAWNFEKFLIGPEGEPPFRRYSR
TFPTINIEPDIKRLKVAI (SEQ ID No.8)

FIG. 22B

hRGMR Sequence:

hCT18626:

[illegible]

FIG. 23A

B. Protein Sequence:

hCP43057:

MIRKKKKRSAPPGPCRSHGPRFATAPAPPPSPFETRPFAWTGMGLRAAPSSAAAAAAAEVQRRRPGLCPPFLELLLLLLLLFSLGLLHA
GDCQQAPCNYQKCTTDFVLSLTHLSNASVDGFSEFCALRAYAGCQRTSKACAGNLVYHSAVLGISDLMSQRNCSKDGPSTSTN
PEVTHDPQRNYSHGAGAREHRRGDQNPSPVLCFGLFDPHLRTKDNFTCKVGEAGWPLIDNNYLSVQTVNVPVPGSSATATNKA
GYPVLLPSHSVKPTCSFPQITIFKAHHECTDQKVYQAVTDDLPAAFVDGTTSGGSDSAKSLRIVERESGHYVEMHARYIGTTVF
VRQVGRYLTLATRMPEDLAMSYEESQDLQVNGCPLSERIIDGGQVSAALHGLSLTRPSLVQAWPGTLETANTQCHEKMPVKDI
YFOSCFDILLTDANETAHAHSALEDVIALHPRKERHIFPSSGNGTPRGSSDLSVLSGLTCLILVFL*

FIG. 23B

Sequences of Tspan 5:**A) Nucleic sequence**

>gi|21264582|ref|NM_005723.2| Homo sapiens tetraspan 5 (TM4SF9), mRNA
CGCCTTTGCCCGAAGCCCGGGGACGAACCGACGGACCGACCGCCTGGCGCACGGACGCGGGCGCTCGCT
TTGTGTTTCGGGGCTAGCGTCGGCGAGGCTTGAGCTTGCAGCGCGCGGCTTCCCTGCTTTCTCGCGGCCA
CCCCGGCTCCGGCGGCCTCGGCGCGCGAGGGGCTGGAGGTGCGGGAGCCGCTCTCCGCCGGTCCGGTCCC
CGCGCGGCTGAGCCCAGGCCGCCAGCGCCGCGGCCCGTGCGGTGTCCCTGAGCTCCTGCTCCCCGCCG
GGCTGCTCCGAGCAACGGTGCTTCGGAGCTCCAACTCGGGCTGCCGGGGCAAGTGTCTTCATGAACCC
AGAGGATGTCCGGAAGCACTACAAGGTCCTGAAGTCAGTTGTTGCATCAAATACTTCATATTTGGCT
TCAATGTCATATTTTGGTTTTTGGGAATAACATTTCTTGAATTGGACTGTGGGCATGGAATGAAAAAG
GAGTTCGTCCAACATCTCTCCATCACCGATCTCGGCGGCTTTGACCCAGTTTGGCTCTTCCTTGTTGG
TGGGAGGAGTGATGTTTATTTTGGGATTTGCAGGGTGCATTGGAGCGCTACGGGAAAACACTTTCCTTC
TCAAGTTTTTTTCTGTGTTTCTGGGAATTATTTTCTTCCTGGAGCTCACTGCCGGAGTTCTAGCATTTG
TTTTCAAAGACTGGATCAAAGACCAGCTGTATTTCTTTATAAACAACAACATCAGAGCATATCGGGATG
ACATTGATTTGCAAAACCTCATAGACTTCACCCAGGAATATTGGCAGTGCTGTGGGGCTTTTGGAGCTG
ATGATTGGAACCTAAATATTTACTTCAATTGCACAGATTCCAATGCAAGTCGAGAGCGATGTGGCGTTC
CATTCTCCTGCTGCACTAAAGATCCCGCAGAAGATGTCATCAACACTCAGTGTGGCTATGATGCCAGGC
AAAAACCAGAAGTTGACCAGCAGATTGTAATCTACACGAAAGGCTGTGTGCCCCAGTTTGAGAAGTGGT
TGCAGGACAATTTAACCATCGTTGCTGGTATTTTTCATAGGCATTGCATTGCTGCAGATATTTGGGATAT
GCCTGGCCCAGAATTTGGTTAGCGATATCGAAGCTGTCAGGGCGAGCTGGTAGACCCCCCTGCAACCGCT
GCTGCAAGACACTGGACAGACCCAGCTTTCGGGACCCTCCCGCGTGCCGAACCTGATCTTCGAGCTGCAT
GGACCTAATCACAGATGCAGCCTGCAGTCTCGCCTAATGGAGCTGCCATTAGGGGAGTGTAATAACTGGG
AAATGCTGCTCACTGACAGAATTAAAAAATAACCAGTATGAAAGTCGTTGCGCCGTGAATCTCT
ACTGTAGCCATGAATTTATGGACAGTTAGATGCTTACCAAAAAAGAAAAAAA (SEQ ID No.11)

FIG. 24A**B) Protein Sequence of Tspan5:**

>gi|21264583|ref|NP_005714.2| (NM_005723) tetraspan 5; tetraspan
TM4SF; tetraspan NET-4; transmembrane 4 superfamily member 9;
transmembrane 4 superfamily, member 8; tetraspanin 5 [Homo sapiens]
MSGKHYKGPEVSCCIKYFIFGFNVIFWFLGITFLGIGLWAWNEKGVLSNISSITDLGGFDPVWLFLVVG
GVMFILGFAGCIGALRENTFLLKFFSVFLGLIFFLELTAGVLAFFVKDWIKDQLYFFINNNIRAYRDDI
DLQNLIDFTQEYWQCCGAFGADDWNLNIYFNCTDSNASRERCVPFSCCTKDPEDVINTQCGYDARQK
PEVDQQIIVIYTKGCVPPQFEKWLQDNLTVAGIFIGIALQLQIFGICLAQNLVSDIEAVRASW (SEQ ID
No.12)

FIG. 24B